

A COMBINATION AND METHOD OF TREATMENT OF CANCER
UTILIZING A COX-2 INHIBITOR AND
A 3-HYDROXY-3-METHYLGLUTARYL-COENZYME-A (HMG-CoA) REDUCTASE
INHIBITOR

CONTINUATION DATA

For purposes of priority, including in the United States of America, this invention is a continuation-in-part of Provisional Applications 60/238,505 and 60/238506 filed October 6, 2000, Provisional Applications 60/243901 and 243,902 filed October 27, 2000, Provisional Application 60/245,592 filed November 17, 2000, Provisional Application 60/264,511 filed January 26, 2001, and Provisional application 60/307689 and Utility Application 09/912703 both filed on July 25, 2001, and PCT/US01/31328 which provisional applications and utility application and other application(s) are incorporated by reference.

SUMMARY OF INVENTION:

The inventors propose a combination of an HMG-CoA reductase inhibitor (also referred to as "HMG-CoA inhibitor(s)"), and COX-2 inhibitor for the treatment of cancer especially prostate cancer and a method of treatment of cancer by that combination, especially prostate cancer. The inventors propose a combination of an HMG-CoA reductase inhibitor, COX-2 inhibitor, and glutathione pathway enhancing and detoxifying compound, particularly cystine, for the treatment of cancer especially prostate cancer and a method of treatment of cancer by that combination, especially prostate cancer. Methods of manufacturing are also claimed. The invention, however, is applicable to cancers generally in mammals and the reference to human biochemistry is not intended to be limiting, but illustrative. The term patient or body or reference to humans is utilized for convenience, but includes all mammalian patients or bodies.

Background:

Traditional cancer treatments have generally used an approach which is focused on directly attacking cells with a propensity to divide. The cancer cell is viewed as a bad cell that must be eliminated. The methods and combinations chosen focus on destruction of the dividing cell, or chemical attack of the cell.

This invention proposes a different methodology. The first premise is to recognize the highly adaptable characteristics and durable biochemistry of the cancer cell from a biochemical

and genetic viewpoint. Many cancer cells are body cells gone awry. The literature solidly suggests that cancer cells in a patient's body have a capability to readapt their functions to adjust to ambient conditions. A patient's body also has an impressive capability to adapt to changing macro-environmental conditions, as well as the micro-environmental conditions in biological chemistry internal to the cell.

Cancer cells, in a genetic or evolutionary sense, are not "bad" cells. Rather, they are efficient cells; in fact, they are highly efficient cells in a certain way. They use relatively less oxygen for the total amount of activity they undertake, and they divide rapidly, enabling them by normal processes of mutation and evolution to adapt their genetic material more quickly. Were the systems and cells in the rest of our bodies equally efficient, we would be greater evolutionary giants than we stand today.

For any attack on cancer cells to be successful, unless they can be physically cut out of the body by surgery, the attack cannot be "too successful." Cancer cells are us, and in a much slower evolutionary way, we are cancer cells. Too much success in damaging cancer cells pharmacologically in the prior art has often been destructive of the host body.

Returning to and illustrating the principle that the body is one large biochemical machine, suppose drops of salt water with colored salt are added to a larger volume of pure water in a container. The body is close to 98% seawater, meaning traditional H_2O water with many other substances and compounds floating in the water. At first the drops would appear whole, but gradually the drops would dissipate so that the entire container might take on a tinge of color. The salt would be dispersed throughout the container so that, once equilibrium was established, all parts of the container had an equal concentration of the salt for each small volume of water. Before that equilibrium was established, the drops of colored water carrying the salt would tend to flow from areas of higher concentration (such as the original drops) to areas of lower concentration in the container (such as the "corners" of the container where there was originally no colored water. That tendency to flow from areas of greater concentration to lesser concentration calls for a resolution of osmotic imbalance generating a pressure gradient and is very important to understanding this invention.

Our bodies are not however, a mere blob of water without structure. Cells are a packet of "sea water" with many compounds in the water surrounded by a membrane. Just like a pile of wet sand full of water will not hold its shape for building a sand castle, but is very strong and can

form a formidable dike if the wet sand is in a bag, the contents of cells in a body, surrounded by a membrane, give the body of humans its structure. Metaphorically, human beings are a standing milieu of tiny piles of sea water in bags called membranes.

On a microscopic scale, the body acts the same way as the earlier described container of salt water. Drops in the form of minute or low concentrations of biologically significant chemicals gradually diffuse throughout our body through links from the membrane bags of sea water in systems of pipes called blood and lymph vessels. Taking advantage of differences in concentration, the blood vessels biochemically "transport" substances either to cells or from cells. Within cells, biochemicals travel by osmosis affected and influenced by biochemical cycles. When cells are short of glucose, the basic fuel product of food, cells have a lower concentration of a substance they need, and if there is a higher concentration of glucose in an adjacent capillary which has a blood cell, some of that glucose flows across the membrane in a complicated biochemical transport mechanism to restore the concentration of glucose in the cell, naturally depleting the concentration in the blood stream.

To complicate the picture in the body context, not all membranes allow all substances to pass. Some are only semi-permeable, allowing only compounds in certain shapes or sizes to pass. For those semi-permeable membranes, if the concentration of compounds on one side of the membrane changes, for instance, increases, then water will flow to that side of the membrane to re-balance the concentration.

Relying on the premise that cancer cells need to divide or replicate (since if they are stable they either pose less danger or are gradually eliminated), the invention takes advantage of that tendency of cancer cell's needs which cause chemicals to flow from areas of greater concentration to those of lesser concentration. First, cancer cells need energy in order to do what they do the most and best, which is to divide or replicate. Energy in a cell is provided by the Krebs cycle. Cancer cells, because they divide frequently, are very sensitive to interference with their energy processes.

Second, when any cell divides, including cancer cells, the bag around the cell which is the membrane has to split into two bags. This presents two problems for the cancer cell. One, the cancer cell needs relatively more cholesterol in order to replicate successfully than a normal cell needs for its normal activities. Two, the membrane is necessarily weakened somewhat as the dividing process occurs and the cell transforms from one cell into two cells like a sandwich

being pulled apart into two halves.

The human body is not completely helpless against cancers. However, cancer cells are relatively good at deceiving or confusing the immune system of our body into believing that the cancer cells are not as bad as they really are, or alternatively, because of rapid replication and evolution, developing defenses against the immune system. Further, as cancer progresses, it damages the body's immune system, including by triggering long-term inflammatory mechanisms.

In total, this invention proposes to use a novel combination to inhibit key biochemical cycles in a way that causes more damage to the cancer cell than to other cells, to decrease long-term inflammation, and to improve and sustain the body's immune system so it can better attack the weakened cancer cells and support the body's remaining essential functions. The inventors propose to selectively modify several biochemical pathways so as not to destroy overall body function, but disproportionately harm cancer cells, to enhance the body's immune system in order that the immune system may attack the cancer cells, and by stressing the cancer cell, to inhibit the cancer cell's normal resistance to immune system function, and to protect the body's normal cells.

The inventors propose a method of treatment of cancer, particularly prostate cancer and pancreatic cancer, by a particular combination of drugs for that purpose which has not been previously proposed for that purpose. The inventors propose a method of treatment of cancer involving a novel combination of drugs which simultaneously slows the cancer but also enables the body's immune system to better attack or fend off the cancer.

The first object of this invention proposes to selectively interfere with the production of cholesterol in two places in a way that impairs the energy cycle of all cells but which normal cells can overcome because they need less energy to survive because they are not dividing, but in a way that has a disproportionate and damaging effect on cancer cells which must replicate, or the cancer will not spread. This object takes advantage of the cancer cell's requirement for cholesterol causing biochemical signaling for cholesterol if not adequate to meet the replicating cancer cell's needs.

A second object is to selectively modify a biochemical cycle that targets inflammatory mechanisms in the body. One of the most damaging aspects of cancer cells is that they trigger an extended inflammatory response in the body. Further, as cancer progresses, it damages the

body's immune system by a number of mechanisms, including the triggering of an extended inflammatory response in the body, which is less efficient in the removal of cancers.

Prostaglandins are some of the most important signals to cause inflammatory responses. The biochemical cycle that we propose to selectively inhibit is an important cycle that converts arachidonic acid to several forms of prostaglandins. That cycle is the cyclooxygenase or COX cycle.

Biochemical cycles have many intermediate steps in them and the intermediate compounds are known as "intermediates." One of those intermediates in the cyclooxygenase cycle is prostaglandin H2 synthase, which has two forms: COX-1 and COX-2. COX-1 is known as a housekeeping substance which helps generate substances that protect the stomach. Ding et al, "Blockade of Cyclooxygenase-2 Inhibits Proliferation and Induces Apoptosis in Human Pancreatic Cancer Cells, vol. 20 AntiCancer Research, 2625-2632 (2000). Aspirin inhibits COX-1 and therefore, because it inhibits a substance that protects the stomach, often has gastrointestinal side effects. Recently, substances have become available that selectively inhibit COX-2 enzymes over COX-1 enzymes. COX-2 enzymes regulate pain, inflammation and fever, i.e. inflammatory mechanisms.

The COX-2 inhibitors in this invention interfere with the transformation of a substance called squalene to cholesterol. There are numerous intermediates from squalene to cholesterol.

Earlier in the biochemical cycle that produces cholesterol is a substance called Acetyl-CoA enzyme. It is converted to an intermediate called mevalonate by an enzyme called 3-hydroxy-3-methylglutamate-CoA reductase ("HMG-CoA"). Recent pharmaceutical advances have produced a number of substances that inhibit the activity of HMG-CoA and slow the production of cholesterol. HMG-CoA inhibitors have been used and are claimed to be used to reduce cholesterol to slow various blood vessel and related heart disease problems which we generally refer to as cardiovascular disease.

A third object of this invention is to utilize the more optimal function of cystine in the pH balance of a normal cell than in the lower pH of a cancer cell. The administration of cystine, enhances the body's immune system benefitting the total body disproportionately to any benefit cystine administration may have for a cancer cell.

In sum, the premise of this invention is that the cancer cells divide rapidly, that they have significant anaerobic glycolytic processes, and that the body is one large biochemical machine in

which we can play to the strength of our body to the detriment of the cancer cell.

The science behind the combination is based on a triad of attacks on the biochemical pathways contributing to cancer cell replication.

Cancer cells must necessarily replicate for a "cancer" to thrive. Attacks on biochemical cycles at points where replication are involved are a favored approach. Cancer cells are particularly vulnerable to interference with lipid cell membrane status and ATP synthesis.

The COX-2 inhibitor interferes with the operation of the cyclooxygenase cycle from which are generated prostaglandins critical in cell division chemistry, and inhibits the "long-term" effects of inflammatory effects. Fosslien, "Biochemistry of Cyclooxygenase (COX)-2 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin. Lab. Sci. 37(5): 431-502 (November 2000).

Tumors and their malignant cancer cells multiply in an exponential growth pattern relative to other body cells. Any retardation of replication will have an exponential effect in slowing cancer growth. Any apoptosis of a cancer cell has a disproportionately exponential effect in retarding cancer. Current treatments such as chemotherapy and radiation therapy which have severe quality of life effects have relied on this disproportionately exponential effect to achieve what benefits those treatments do achieve for extending the life of patients.

This invention has the further benefit as distinct from prior art of accomplishing its benefits with substantially less interference with quality of life than chemotherapy and radiation therapy(ies) in particular.

Subsequent to earlier provisional applications, a citation is made in Drug Facts and Comparisons, 55th ed. 2001 at KU-16 (Publ. by Facts & Comparisons 2000) to a pending trial of Ubiquinone under a trade name of Ubigel by Gel-Tec. Ubiquinone or CoQ-10 administration is not likely have the benefits of the present invention because it is proposed to be administered by macroadministration to the entire organism, either orally or intravenously or in the general vicinity of the tumor area.

By contrast to such effort at macroadministration, this invention as proposed in the prior provisional application proposes virtual microadministration. This is a unique aspect of this invention and an important concept behind the invention. The inventors propose that one of the dilemmas of cancer therapy is to deliver the needed dose to the right place and minimize harm when the therapy is not in the right place.

The inventors believe that the most optimal treatments involve the utilization of the biochemical physiologic machine of the body, and preferably of the individual cell, to construct, manufacture and adjust the individual cell chemistry to achieve the desired object: in the case of the cancer cell or other afflicted and undesired cell, to disrupt its mechanisms of replication, primarily by focusing on the energy mechanism of the cell with the corollary result of interfering with membrane synthesis and cell replication, and in many instances, as the cell struggles to reach homeostasis, inducing apoptosis. Simultaneously, the remaining desired and normal cells must be reinforced to meet the threat of the cancer and to resist the side effects of the treatment that interfere with normal cell operation, which is why cystine, in addition to its increase in TH1 to Th2 ratio, achieves notable benefit despite literature suggesting to the contrary. See, for example, "Clinical Oncology" (Amer. Cancer Society 2001) at 186 (discourages medical practitioners from glutathione pathway enhancement); Volies and Golomb, "Oncological Therapies" (Springer 1999) at 126 in the selection by Ratain, Ewe, Suede, entitled Cancer Chemotherapy at 36-100. As another example, one of the clear benefits of the selective COX-2 inhibitor is that COX-1 isoenzymes have what has been characterized as general housekeeping functions generally ameliorative to bodily health. Aspirin, a classic COX-2 inhibitor, also inhibits COX-1, thereby achieving anti-inflammatory effect, for which aspirin is well-known, at the cost of beneficial aspects of COX-1 isoenzymes. Thus, a selective COX-2 inhibitor is important in the invention.

Lipoic acid can be an adjunct to cystine in the invention. Lipoic acid also has a disulfide bond as does cystine. That disulfide bond can be separated and the sulphur protonated with hydrogen. Thus, lipoic acid can reinforce the benefits of cystine. For instance, a ras oncogene generates a ras protein. "The transforming (carcinogenic) activity of the ras oncogene is lost when isoprenylation of the Ras protein is blocked, stimulating interest in identifying inhibitors of this posttranslational modification pathway for use in cancer chemotherapy." Nelson, Cox, Lehninger Principles of Biochemistry at 1054 (3rd ed. 2000 Worth Publishers). Because the same thiol group on a Ras protein is the thiol group found on cysteine or available on separation of the disulfide bridge of cystine or lipoic acid, cystine and to a lesser degree, lipoic acid, act as competitive inhibitor of isoprenylation of the thiol group on the Ras protein thereby disabling its ability to stabilize in a membrane and blocking its carcinogenic activity. A typical dose would be 300 mg oral per day.

1 The inventors also note the need for and claim a composition potentially including
2 Selenium, and the method of administration potentially including Selenium, if a
3 therapeutic window of Selenium in a patient is not present. See, Brooks and Nelson,
4 Cancer Prevention and Control, Chemoprevention of Cancer at 369 (Marcel Dekker
5 1995). Selenium can be toxic, but there does need to be an adequate level of Selenium.
6 The patient should be monitored and Selenium supplement given to achieve a therapeutic
7 window for Selenium level to achieve the desired effect of allowing normal functioning
8 of the glutathione pathway and maintaining integrity. In a normal healthy male, the
9 adequate level is approximately 70 micrograms/70 kg of weight. The preferred mode
10 would be a supplement in sequence with cystine administration, but a dose of any part of
11 the invention could include Selenium. The method of treatment could include a
12 sequential or simultaneous dose with either the cystine or the COX-2 inhibitor or both.
13 However, toxic levels of selenium must be avoided. Thus, adequate level means only
14 adequate level.

15 The inventors recognize that vitamin E deficiency may allow oxidative stress and
16 the inventors claim that like Selenium, the level of Vitamin E must be maintained, but
17 normal vitamin E levels per se do not strengthen the immune system sufficiently to deter
18 metastasis.

19 Vitamin C also has antioxidative properties, and again the inventors recognize
20 that vitamin C deficiency may allow oxidative stress and the inventors claim that like
21 Selenium and Vitamin E, the level of Vitamin C must be maintained, but normal vitamin
22 C levels per se do not strengthen the immune system sufficiently to deter metastasis.
23 Vitamin C protects and maintains the redox balance of the cell.

24 Adequate levels of Vitamin C and Vitamin E means, in this invention, for a
25 cancer patient, approximately three times the recommended daily allowance as set out by
26 the American Dietetic Association or the U.S. Dept. of Agriculture as published from
27 time to time.

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29 Discussion of certain specific patent and literature art:

30 One patent, Winokur, PCT Appl. US98/21901, filed 16 Oct. 1998, published as
31 WO99/20110 entitled "Combination Therapy for Reducing the Risks Associated with

1 Cardio and Cerebrovascular Disease”, and a corresponding U.S. Patent 6,245,797, claims
2 a combination of a COX-2 inhibitor with an HMG-CoA inhibitor for treating, preventing,
3 and/or reducing the risk of atherosclerosis and atherosclerotic disease events and a
4 method of using a COX-2 inhibitor with an HMG-CoA inhibitor for treating, preventing,
5 and/or reducing the risk of atherosclerosis and atherosclerotic disease events. Another
6 patent, Nichtberger, U.S. Pat. 6,136,804, October 24, 2000, entitled “Combination
7 therapy for treating, preventing, or reducing the risks associated with acute coronary
8 ischemic syndrome and related conditions” proposes the utilization for an antiplatelet
9 agent in combination with a therapeutically effective amount of a COX-2 inhibitor to
10 treat, prevent or reduce the risk of acute coronary ischemic syndrome, thrombosis, and
11 related vascular problems.

12 Certain literature has suggested that COX-2 inhibitors may have efficacy toward
13 certain cancers. A review article sets out a good summary of COX-2 inhibitors. Fosslien
14 “Biochemistry of Cyclooxygenase (COX)-2 Inhibitors and Molecular Pathology of COX-
15 2 in Neoplasia,” Crit. Rev. in Clin. Lab. Sci. 37(5): 431-502 (2000). In unrelated
16 research, COX-2 inhibitors were reported to be inhibiting certain cancers, particularly
17 familial adenomatous polyposis. See, 319 (7218) British Medical Journal 1155 (Oct. 30,
18 1999). COX-2 inhibitors, in that instance, celecoxib, a COX-2 inhibitor manufactured by
19 G.D.Searle, and sold under the brand name Celebrex, had caused a reduction in
20 adenomatous polyps which are a virtual guarantor of cancer of the colon if left untreated.
21 Cyclooxygenase-2 had been implicated in colorectal cancer and colonic tumorigenesis.
22 See, “The Relationship Between Cyclooxygenase-2 Expressions and Colorectal Cancer”,
23 282(13) J. Amer. Med. Ass’n:1254-1257 (Oct. 6, 1999).

24 Both celecoxib and rofecoxib are suggested to have similar effects. See, Vol.
25 56(2) Amer. J. of Health-System Pharmacy: 106-107 (Jan. 15, 1999). Unfortunately,
26 like many (nonsteroidal anti-inflammatory drugs (NSAIDs), the COX-2 inhibitors are felt
27 to cause a range of gastrointestinal problems.

28 Based on the pharmaceutical product description of Merck for simvastatin, which
29 description is adopted herein and attached for reference, and which drug is marketed as
30 ZOCOR, a registered trademark of Merck, simvastatin functions in a similar way to
31 lovastatin, another drug marketed by Merck under the registered trademark of

1 MEVACOR, the pharmaceutical product description for which is adopted herein and
2 attached for reference. Both are derived from *aspergillus terreus*.

3 Certain literature has suggested that HMG-CoA inhibitors may have efficacy
4 toward certain cancers. Based on an article entitled, "Caspase-7 is Activated During
5 Lovastatin Induced Apoptosis of the Prostate Cancer Cell Line LNCaP" 58(1) Cancer
6 Research: 76-83 (1998), and a second article "Inhibition of the 3-hydroxy-
7 3methylglutaryl-coenzyme A reductase pathway Induces p53-independent
8 Transcriptional Regulation of p21 (WAF1/CIP1) in human prostate carcinoma cells",
9 273(17) J. Biol. Chem.:10628-23, (1998), lovastatin had therapeutic value in treating
10 prostate cancer. Patients to whom were administered lipid lowering/modifying drugs
11 such as lovastatin were suggested to be more cancer-free than those using bile acid-
12 binding resins. See, 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors and
13 the Risk of Cancer: A Nested Case-Control Study, 160(5) Archives of Internal Med:
14 2363-2368 (2000).

15 "Therapeutic Approaches to Bone Diseases [Bone Remodeling and Repair: Review],"
16 Science, 289(5484), Sept. 1, 2000:1508-1514.

17 No patent or literature suggests that the substances be combined to treat cancer
18 nor is the synergistic effect set forth in this specification suggested or described.

19 No patent or literature suggests the preferred embodiment that a COX-2 inhibitor
20 be combined with an HMG-CoA inhibitor to retard cancer and be further combined with
21 a glutathione-cycle enhancing compound such as cystine, cysteine, or N-acetyl-cysteine,
22 also called NAC, to improve immune system competency to further retard cancer.

23 No literature suggests another preferred embodiment: using a COX-2 inhibitor
24 and HMG-CoA inhibitor set forth in this invention to retard cancer.

25 Reduction to practice:

26 The combination of a selective COX-2 inhibitor and an HMG-CoA reductase
27 inhibitor exhibits the unexpected property of enabling management of cancer. This has
28 been demonstrated in two specific instances. Both patients were diagnosed with Stage 4
29 metastatic cancer and were refractory to other treatments. The first patient had prostate
30 cancer and showed a PSA (prostate specific antigen-a widely accepted marker of prostate
31 cancer activity) of 71 according to the patient. The patient was placed on a regimen of

1 VIOXX and MEVACOR, and has survived with good quality of life such as mowing his
2 lawn, steady weight, and the like while the patient's PSA fell from tests conducted by one
3 of the inventors to less than 2.5 with scan-documented lack of progression. A second
4 patient diagnosed with pancreatic cancer which was also refractory to other treatment was
5 placed on a regimen of VIOXX and MEVACOR with a whey supplement containing
6 cystine and survived approximately four months and initially gained some weight since
7 first presenting while sustaining a reasonable quality of life until death. Pancreatic cancer
8 is one of the most intractable cancers known and any success with pancreatic cancer is
9 surprising in light of existing literature and art.

10 Pharmacological compounds in this invention:

11 The science behind the combination is based on a triad of attacks in the
12 biochemical cycles contributing to cancer cell replication.

13 Cancer cells must necessarily replicate for a "cancer" to thrive. Attacks on
14 biochemical cycles at points where replication are involved are a favored approach.
15 Cancer cells are particularly vulnerable to interference with lipid cell membrane status
16 and ATP synthesis.

17 This invention proposes not only attack with a COX-2 inhibitor to interfere with
18 the cyclooxygenase pathway, but by combination with an HMG-CoA reductase inhibitor,
19 a statin, including simvastatin or lovastatin, focuses on another cycle, the formation of
20 polyisoprenoids, particularly cholesterol.

21 The invention claims the use of selective COX-2 inhibitor, including rofecoxib or
22 celecoxib, but the principles stated are generally applicable to all selective COX-2
23 inhibitors. The meaning and definition of Cyclooxygenase-2 inhibitor ("COX-2
24 inhibitor" or "selective COX-2 inhibitor") in this invention shall include the following in
25 this paragraph: all of the compounds and substances beginning on page 8 of Winokur
26 WO99/20110 as members of three distinct structural classes of selective COX-2 inhibitor
27 compounds, and the compounds and substances which are selective COX-2 inhibitors in
28 Nichtberger, U.S. Pat. 6,136,804, October 24, 2000, entitled "Combination therapy for
29 treating, preventing, or reducing the risks associated with acute coronary ischemic
30 syndrome and related conditions", and the compounds and substances which are selective
31 COX-2 inhibitors in Isakson et al, PCT application WO/09641645 published 27

December 1996, filed as PCT/US 9509905 on 12 June 1995, entitled "Combination of a Cyclooxygenase-2 Inhibitor and a Leukotriene B4 Receptor Antagonist for the Treatment of Inflammations," and in Waldstreicher, WO 01/45698, filed 18 December 2000, published June 28, 2001 entitled "Combination Therapy for Treating Neurodegenerative Disease." Because the common names of some of the selective COX-2 inhibitor compounds are not given in Winokur, PCT WO99/20110, Nichtberger, U.S. Pat. 6,136,804, Isakson, PCT WO/09641645, and Waldstreicher, WO01/45698, the meaning of COX-2 inhibitor in this invention includes compounds that are selective COX-2 inhibitors, such as NS398 and DFU (see, YERGEY, JAMES A., et al., "In Vitro Metabolism of the COX-2 Inhibitor DFU, Including a Novel Glutathione Adduct Rearomatization," Drug Metabolism and Disposition 29(5): 638-644 (The American Society for Pharmacology and Experimental Therapeutics 2001), also known as 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone. The meaning of COX-2 inhibitor in this invention includes compounds that are selective COX-2 inhibitors referenced in Fosslein, "Biochemistry of Cyclooxygenase (COX)-2 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin. Labor. Sci. 37(5):431-502 (CRC Press LLC 2000). The meaning of COX-2 inhibitor in this invention also includes rofecoxib, and celecoxib, marketed as VIOXX and CELEBREX by Merck and Searle/Pfizer respectively. Rofecoxib is discussed in Winokur, WO99/20110 as compound 3, on p.9. Celecoxib is discussed as SC-58635 in the same reference, and in T. Penning, Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib)", J. Med. Chem. 1997 Apr 25; 40(9): 1347-56. The meaning of COX-2 inhibitor in this invention also includes SC299 referred to as a fluorescent diaryloxazole. C. Lanzo et al, "Fluorescence quenching analysis of the association and dissociation of a diarylheterocycle to cyclooxygenase-1 and cyclooxygenase-2: dynamic basis of cyclooxygenase-2 selectivity", Biochemistry 2000 May 23 vol. 39(20):6228-34, and in J. Talley et al, "4,5-Diarylloxazole inhibitors of cyclooxygenase-2 (COX-2)", Med. Res. Rev. 1999 May; 19(3): 199-208 . The meaning of COX-2 inhibitor in this invention also includes valdecoxib, See, "4-[5-Methyl-3-phenylisoxazol-1-yl]benzenesulfonamide,

1 Valdecoxib: A Potent and Selective Inhibitor of COX-2", J. Med. Chem. 2000, Vol. 43:
2 775-777, and parecoxib, sodium salt or parecoxib sodium, See, N-[[[(5-methyl-3-
3 phenylloxazol-4yl)-phenyl]sulfonyl]propanimide, Sodium Salt, Parecoxib Sodium: A
4 Potent and Selective Inhibitor of COX-2 for Parenteral Administration", J. Med. Chem.
5 2000, Vol. 43: 1661-1663. The meaning of COX-2 inhibitor in this invention also
6 includes the substitution of the sulfonamide moiety as a suitable replacement for the
7 methylsulfonyl moiety. See, J. Carter et al, Synthesis and activity of sulfonamide-
8 substituted 4,5-diaryl thiazoles as selective cyclooxygenase-2 inhibitors", Bioorg. Med.
9 Chem. Lett 1999 Apr. 19:Vol. 9(8): 1171-74, and compounds referenced in the article
10 "Design and synthesis of sulfonyl-substituted 4,5-diarylthiazoles as selective
11 cyclooxygenase-2 inhibitors", Bioorg. Med. Chem. Lett 1999 Apr. 19:Vol. 9(8): 1167-
12 70. The meaning of this invention includes a COX-2 inhibitor, NS398 referenced in two
13 articles: Attiga et al, "Inhibitors of Prostaglandin Synthesis Inhibit Human Prostate
14 Tumor Cell Invasiveness and Reduce the Release of Matrix Metalloproteinases", 60
15 Cancer Research 4629-4637, August 15, 2000, and in "The cyclooxygenase-2 inhibitor
16 celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells
17 independently of Bcl-2," Hsu et al, 275(15) J. Biol. Chem. 11397-11403 (2000). The
18 meaning of COX-2 inhibitor in this invention includes the cyclo-oxygenase-2 selective
19 compounds referenced in Mitchell et al, "Cyclo-oxygenase-2: pharmacology, physiology,
20 biochemistry and relevance to NSAID therapy", Brit. J. of Pharmacology (1999) vol.128:
21 1121-1132, see especially p. 1126. The meaning of COX-2 inhibitor in this invention
22 includes so-called NO-NSAIDs or nitric oxide-releasing-NSAIDs referred to in L.
23 Jackson et al, "COX-2 Selective Nonsteroidal Anti-Inflammatory Drugs: Do They Really
24 Offer Any Advantages?", Drugs, June, 2000 vol. 59(6): 1207-1216 and the articles at
25 footnotes 27, and 28. Also included in the meaning of COX-2 inhibitor in this invention
26 includes any substance that selectively inhibits the COX-2 isoenzyme over the COX-1
27 isoenzyme in a ratio of greater than 10 to 1 and preferably in ratio of at least 40 to 1 as
28 referenced in Winokur WO 99/20110, and has one substituent having both atoms with
29 free electrons under traditional valence-shell-electron-pair-repulsion theory located on a
30 cyclic ring (as in the sulfylamine portion of celecoxib), and a second substituent located
31 on a different ring sufficiently far from said first substituent to have no significant

1 electron interaction with the first substituent. The second substituent should have an
2 electronegativity within such substituent greater than 0.5, or the second substituent
3 should be an atom located on the periphery of the compound selected from the group of a
4 halogen F, Cl, Br or I, or A group VI element S or O. Thus for purposes of this last
5 included meaning of a COX-2 inhibitor, one portion of the COX-2 inhibitor should be
6 hydrophilic and the other portion lipophilic. Also included as a COX-2 inhibitor are
7 compounds listed at page 553 in Pharmacotherapy, 4th ed: A Pathophysiologic
8 Approach, Depiro et al (McGraw Hill 1999) including nabumetone and entodolac.
9 Recognizing that there is overlap among the selective COX-2 inhibitors set out in this
10 paragraph, the intent of the term COX-2 inhibitor is to comprehensively include all
11 selective COX-2 inhibitors, selective in the sense of inhibiting COX-2 over COX-1. The
12 package inserts for rofecoxib and celecoxib are attached and adopted herein by reference.
13 The inventors add to the class of COX-2 inhibitors useful in the invention the drug
14 bearing the name etoricoxib referenced in the Wall Street Journal, December 13, 2000
15 manufactured by Merck. See, also, Chauret et al, "In vitro metabolism considerations,
16 including activity testing of metabolites, in the discovery and selection of the COX-2
17 inhibitor etoricoxib (MK-0663)," Bioorg. Med. Chem. Lett. 11(8): 1059-62 (Apr 23,
18 2001). Another selective COX-2 inhibitor is DFU [5,5-dimethyl-3-(3-fluorophenyl)-4-(4-
19 methylsulphonyl) phenyl-2(5H)-furanone] referenced in Yergey et al, Drug Metab.
20 Dispos. 29(5):638-44 (May 2001). The inventors also include as a selective COX-2
21 inhibitor flavanolignanes (sometimes also called flavonoids) which have selective COX-2
22 inhibitory activity over COX-1 inhibitory activity, including the flavanoid antioxidant
23 silymarin itself, and an active ingredient in silymarin, silybinin, which demonstrated
24 significant COX-2 inhibition relative to COX-1 inhibition. The silymarin also showed
25 protection against depletion of glutathione peroxidase. Zhao et al, "Significant Inhibition
26 by the Flavonoid Antioxidant Silymarin against 12-O-tetradecanoylphorbol 13-acetate-
27 caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase 2 and
28 interleukin-1 alpha expression in SENCAR mouse epidermis: implications in the
29 prevention of stage I tumor promotion," Mol. Carcinog. Dec. 1999, Vol 26(4):321-33
30 PMID 10569809. Silymarin has been used to treat liver diseases in Europe. Bombardelli
31 et al, U.S. Pat. 5,912,265, June 15, 1999, and Bombardelli et al, U.S. Pat. 6,218,369,

1 April 17, 2001 list compounds having similar characteristics and related to silymarin
2 intended to be included as COX-2 inhibitors in this invention, including silymarin,
3 silibinin, silidianin, silicristin, dehydrosilybin, and phospholipid complexes of one of
4 those flavolignanes. The minimum recommended dose in the therapeutic window is 200-
5 250 mg/day of those compounds.

6 The term COX-2 inhibitor includes all pharmaceutically acceptable salts for the
7 selective COX-2 inhibiting compound selected. Examples of such salt forms of COX-2
8 inhibitors include but are not limited to salts derived from inorganic bases including
9 aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic
10 salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the
11 ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from
12 pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary,
13 and tertiary amines, substituted amines including naturally occurring substituted amines,
14 cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline,
15 N,N-dibenzylethylenediamine, diethylamide, 2-diethylaminoethanol, 2-
16 dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-
17 ethylpiperidine, glutamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine,
18 methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purine,
19 theobromine, triethylamine, trimethylamine, tripropylamine, troethamine, and the like.

20
21 The HMG-CoA reductase inhibitor claimed in this invention is lovastatin or
22 simvastatin or cholestin which are compounds related to aspergillus terreus. The
23 principles of this invention are generally applicable to all statins. The meaning and
24 definition of a 3-hydroxy-3-methylglutaryl-Coenzyme-A reductase inhibitor ("HMG-
25 CoA inhibitor") in this invention is any selective, competitive inhibitor of HMG-CoA
26 reductase, the rate-limiting enzyme that converts HMG-CoA into mevalonate, generally
27 referred to as cholesterol-lowering statins, and includes

- 28 1) lovastatin, marketed under the trademark MEVACOR by Merck, and described,
29 among other places in U.S. Pat. 4,231,938,
30 2) simvastatin, marketed under the trademark ZOCOR by Merck, and described, among
31 other places in U.S. Pat. 4,444,784,

1 3) pravastatin, marketed under the trademark PRAVACOL by Bristol-Myers-Squibb, and
2 described, among other places, in U.S. Pat. 4,346,227,
3 4) atorvastatin calcium, marketed under the name LIPITOR by Parke-Davis, and
4 described, among other places, in U.S. Pat. 5,273,995,
5 5) cerivastatin sodium, marketed under the name BAYCOL, by Bayer, and described,
6 among other places, in U.S. Pat. 5,177,080, and
7 6) fluvastatin sodium, marketed under the name LESCOL, by Novartis Pharmaceuticals,
8 and described, among other places, in U.S. Pat. 5,354,772.

9 The term HMG-CoA inhibitor (used as shorthand for and also referred to as
10 "HMG-CoA reductase inhibitor") further includes all HMG-CoA reductase inhibitors
11 described in Winokur, PCT Appl. US98/21901, filed 16 Oct. 1998, published as
12 WO99/20110 entitled Combination Therapy for Reducing the Risks Associated with
13 Cardio and Cerebrovascular Disease," and the compounds and substances which are
14 HMG-CoA inhibitors in Nichtberger, U.S. Pat. 6,136,804, October 24, 2000, entitled
15 "Combination therapy for treating, preventing, or reducing the risks associated with acute
16 coronary ischemic syndrome and related conditions." The meaning of HMG-CoA
17 inhibitor in this invention shall include the compounds and substances referenced and
18 incorporated into Winokur WO99/20110 by reference to art therein, and the compounds
19 and substances referenced and incorporated into Nichtberger, U.S. Pat. 6,136,804,
20 October 24, 2000, by reference to art therein. Compactin is also described as a fungi
21 derived competitive inhibitor of HMG-CoA reductase. Lehninger, Principles of
22 Biochemistry (3rd ed. 2000) at 811. An HMG-CoA reductase inhibitor, with the natural
23 structure of lovastatin identical to the synthetic structure of lovastatin, can also be
24 isolated from red rice yeast or the rice in sufficient quantity and is an HMG-CoA
25 reductase inhibitor. The red rice yeast is found as cholestin or cholestol and is available
26 on the Internet from a variety places including China Beijing Jingxin Biochemical
27 Products Factor, Linxiao Rd. S., Daxing Count, Beijing, PRC or its U.S. agent PHC
28 Resources, Inc., 77 Milltown Rd., East Brunswick, NJ 08816. The red rice yeast is
29 referred to in an FDA warning letter of May 8, 2001 to Maypro Industries available at
30 www.fda.gov/foi/warning_letters/g1249d.pdf.

31 Based on the pharmaceutical product description of Merck for simvastatin, which

1 description is adopted herein and attached for reference, and which drug is marketed as
2 ZOCOR, a registered trademark of Merck, simvastatin functions in a similar way to
3 lovastatin, another drug marketed by Merck under the registered trademark of
4 MEVACOR, the pharmaceutical product description for which is adopted herein and
5 attached for reference. Both are derived from *aspergillus terreus*.

6 Recognizing that there is overlap among the HMG-CoA inhibitors set out in this
7 paragraph and in the list of six HMG-CoA inhibitors set forth above, the intent of the
8 term HMG-CoA inhibitor is to comprehensively include all HMG-CoA reductase
9 inhibitors.

10 The term HMG-CoA inhibitor encompasses the pharmaceutically acceptable salts
11 of HMG-CoA inhibitor selected. The invention includes pharmaceutically active salts of
12 an HMG-CoA inhibitor, which may include non-toxic salts of the compounds employed
13 in this invention which are generally prepared by reacting the free acid with a suitable
14 organic or inorganic base. Examples of salt forms of HMG-CoA reductase inhibitors
15 may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate,
16 bisulfate, bitartrate, borate, bromide, calcium, camsylate, carbonate, chloride, citrate,
17 dihydrochloride, edentate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate,
18 glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide,
19 hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laureate,
20 malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate,
21 mutate, napsylate, mitrate, oleate, oxalate, pamaote, palpitrate, panthothenate,
22 phosphate/diphosphate, polygalacturonate, potassium, sodium, stearate, subacetate,
23 succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate. The principles
24 are also applicable to the inclusion of an additional ingredient, namely an edible resin that
25 binds bile acids and prevents their reabsorption from the intestine, though this is not the
26 preferred mode. Lehninger, Principles of Biochemistry (3rd ed. 2000) at 811.

27 Ester derivatives of the above described compounds included HMG-CoA
28 inhibitors may act as prodrugs which, when absorbed into the bloodstream of a warm-
29 blooded animal, may cleave in such a manner as to release the drug form and permit the
30 drug to afford improved therapeutic efficacy.

31 The package inserts for COX-2 inhibitors and HMG-CoA inhibitors attached to

1 the provisional application 60/245,592 and the description in the patents and methods in
2 those patents related to the selective COX-2 inhibitors and HMG-CoA inhibitors are
3 adopted by reference.

4 Cystine will be used as included in, and as a generic reference to glutathione
5 pathway enhancing and detoxifying compounds in this description. Such compounds
6 include the following in this invention:

7 Cystine is (3,3'-dithiobis [2-aminopropanoic acid]). Cystine is readily reduced to
8 cysteine. Cystine is present in most mammalian hair and keratin.

9 Cysteine is 2-amino-3-mercapto propanoic acid. It is readily converted by
10 oxiorreduction to cystine. It is a constituent of glutathione and abundantly present in the
11 metallothioneines.

12 Cystine in the body-useful form as L-cystine is available from Spectrum
13 Chemical Mfg. Corp. 14422 S. San Pedro St., Gardena, California 90248, and N-acetyl
14 cysteine is also available there.

15 Cystine, cysteine, and N-Acetyl cysteine and pharmaceutically acceptable salts,
16 including the pharmaceutically active forms described in Kozhemyakin et al, published
17 by WIPO as WO 00/031120, PCT/RU99/00453, filed internationally on 19 Nov. 1999,
18 "Hexapeptide with the Stabilized Disulfide Bond and Derivatives Thereof Regulating
19 Metabolism, Proliferation, Differentiation and Apoptosis," will all collectively be
20 referred to as cystine in this invention. Other glutathione pathway enhancing compounds
21 understandable to one of ordinary skill in the art which are encompassed in the term NAC
22 are stable forms of compounds that enhance the glutathione pathway, the substituents of
23 which are suggested in Kozhemyakin et al, Hexapeptide with the Stabilized Disulfide
24 Bond and Derivatives thereof Regulating Metabolism, Proliferation, Differentiation and
25 Apoptosis published as WO 00/31120, June 2, 2000. Included in the term NAC is also
26 any therapeutically beneficial sulfur donating compound, including ebselen, which
27 interacts with the glutathione pathway. The invention contemplates in the term NAC
28 undenatured whey protein products designed to have enhanced cystine concentration as
29 well as protein products which contain cysteine and cystine. They can be in the form of
30 food products. Immunocal (a Registered Trademark of a product manufactured by
31 Immunotec, Montreal Canada). Immunocal ® undenatured whey protein has the added

1 advantage of providing the cysteine in the disulfide form, called cystine. 80% of the
2 circulating cysteine in the body is in the form of cystine. Cystine is readily absorbed into
3 cells and has been demonstrated to be preferred by certain cells such as astrocytes
4 (Kranich O et al Glia, 22(1):11-8 1998).

5 The addition of cystine, cysteine, N-acetyl cysteine, or the pharmaceutically
6 acceptable salt of those substances yields another effect in this invention not facially
7 evident from the independent properties of the basic components of the invention
8 (hereafter each substance or a pharmaceutically acceptable salt is referred to as a
9 "cystine"). Administration of a cystine family member, preferably cystine, which has the
10 best and most rapid upload into the glutathione pathway and better storage capability by
11 the body, or N-acetyl cysteine, enhances the immune system competency of the patient.

12 In individuals on prophylactic antibiotic therapy for presumed exposure to anthrax
13 the NAC can be continued for extended periods with oral ingestion of NAC or a cystine
14 source such as undenatured whey protein such as Immunocal (a Registered Trademark of
15 a product manufactured by Immunotec, Montreal Canada). Immunocal ® undenatured
16 whey protein has the added advantage of providing the cysteine in the disulfide form,
17 called cystine. 80% of the circulating cysteine in the body is in the form of cystine.
18 Cystine is readily absorbed into cells and has been demonstrated to be preferred by
19 certain cells such as astrocytes (Kranich O et al Glia, 22(1):11-8 1998). Lipoic acid can
20 be an adjunct to the cystine.

21 All of these cystine and cystine-like compounds function as a glutathione pathway
22 enhancing and detoxifying compound. They have the additional benefit of ameliorating
23 the negative renal, hepatic and gastric effects of COX-2 inhibitors and HMG-CoA
24 inhibitors, both as a combination and individually. The enhancement of the glutathione
25 level and pathway has a second important and unexpected effect. The avoidance of a
26 glutathione deficiency steers the patient to have a higher Th-1 response to Th-2 response
27 ration that the patient would have with any glutathione deficiency. Peterson, J. et al,
28 "Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response
29 patterns," Vol 95(6), Proceedings Nat'l Acad. Sci. USA p. 3071-76 (Mar. 17, 1998).
30 This enhancement is independent of, but corollary to the combination of the COX-2 and
31 HMG-CoA inhibitor.

1
2 DESCRIPTION OF INVENTION:

3 The preferred mode of invention without limiting its use or use of pharmaceutical
4 equivalents to those described herein is to administer a therapeutic dose of a
5 cyclooxygenase-2 inhibitor, namely VIOXX (a registered trademark of Merck Co. for a
6 drug formally known as rofecoxib) or CELEBREX (a registered trademark of Searle and
7 Pfizer for a drug formally known as celecoxib) (both referred to as a "COX-2 inhibitor"),
8 in combination with a therapeutic dose of a 3-hydroxy-3-methylglutaryl-Coenzyme-A
9 reductase inhibitor, namely with Mevacor (a registered trademark of Merck Co. for a
10 drug formally known as lovastatin), or ZOCOR (a registered trademark of Merck Co. for
11 a drug formally known as lovastatin) or cholestin (all referred to as "HMG-CoA
12 inhibitor") starting with the minimum initial recommended doses of each drug on the
13 package inserts attached to provisional application 60/245,592. This mode is therefore a
14 COX-2 inhibitor beginning with an HMG-CoA inhibitor in the minimum doses for each.
15 For patients who have advanced prostate cancer whose PSA does not respond to the
16 combination, the dosage should be increased in step wise fashion to the maximum dose in
17 the therapeutic window. The preferred mode of so doing is to monitor the patient each
18 six weeks. A person of ordinary skill in the medical arts can apply the regimen described
19 in this specification.

20 The inventors suggest measuring at least cholesterol level and isoprostane level.
21 If a patient's cholesterol level is decreasing, then the HMG CoA inhibitor is affecting
22 cholesterol synthesis. If isoprostane levels are rising, then the COX-2 inhibitor should be
23 having an effect. The lack of change in one or the other suggests that the medication to
24 achieve the desired metabolic pathway effect should be adjusted.

25 Another way to test for effectiveness and enable dosage adjustment is to test
26 cytokine levels. Once at least two inflammatory response markers show therapeutic
27 change then the combination should be having an effect. The preferred markers include
28 upregulation of IL-12 and downregulation of IL-10. "Specific inhibition of
29 cyclooxygenase restores anti-tumor reactivity by altering balance of IL-10 and IL-12
30 synthesis", J. Immunol 2000 vol 164(1) :361-370 [increased COX-2 expression increases
31 PGE-2 which induces IL-10; accordingly, use of COX-2 inhibitor leads to down-

- 1 regulation of IL-10; also observed concomitant upregulation of IL-12]. Testing of
- 2 cytokines involves the use of ELISA assays to determine cytokine levels.
- 3 Chemoluminescence tests are also used for certain interleukins. Other useful
- 4 inflammatory response markers that may be tested include:

5

6

Test/	FactorName/range	Brief description
CRP	C-reactive protein	General inflammatory response marker, downregulation indicates amelioration of inflammatory response mechanism
IL-10	Interleukin-10 ED ₅₀ =0.5 ng-1ng/mL	Potent blocker of activation of cytokine synthesis and several accessory functions of macrophages; produced in CD4+ T cells and T cell clones, and other cells; downregulation indicates lessened interference with cytokine synthesis of cytokines needing upregulation and lessened macrophage activity interference
IL-2	Interleukin-2 0.0-4.0 pg/mL	Activates lymphocytes, potent stimulator of cytokine activated killer cells (LAK's) which demonstrate enhanced MHC non-restricted cytotoxicity. Used for renal cell CA-encourage Tc1 activity
IL-6	Interleukin-6 0.0-149 pg/mL	Involved in T-cell activation; in nesting cells induce the expression of receptors for T-cell growth factor. Very important in inducing B-cells to differentiate into antibody-forming cells. In liver, it stimulates production of acute phase proteins. Growth factor for multiple myeloma
IL-8	Interleukin-8 0.0-70 pg/mL	Proinflammatory cytokine released from range of cells including monocytes, endothelial cells, epithelial cells, hepatocytes, fibroblasts and chondrocytes
IL-12	Interleukin-12 Range 0.7pg/mL-7000pg/mL	Potent initial stimulus for T-and Nk-cell, IFN(IFN=interferon)- γ production. May encourage Tc1 generation. Potentiates NK cell to release IFN-8. Works in a manner complementary to IL-10; increase in level compared to baseline indicates potential for increased cell-mediated response

TNF	Tumor Necrosis Factor 0.0-4.9 pg/mL	Activates macrophage (mφs) and neutrophils
IFN-γ	Interferon-gamma 0.0-1.5pg/mL	Encourages Tc1 generation role in early phase of immune response including antiviral and antiproliferative properties
IFN-α	Interferon-alpha 0.0-1.5 pg/mL	Induces IL-2 and can be used to switch Th cells from a Th2 to a Th1 profile
ECP	Eosinophilic cationic protein 1.5-5.5mg/mL	Potent indicator of eosinophilic degranulation resulting in a wide range of inflammatory conditions: autoimmune disease, bronchial asthma, parasitic infections, viral infections
IL-10	Interleukin-10 ED ₅₀ =0.5 ng-1ng/mL	Potent blocker of activation of cytokine synthesis and several accessory functions of macrophages; produced in CD4+ T cells and T cell clones, and other cells; downregulation indicates lessened interference with cytokine synthesis of cytokines needing upregulation and lessened macrophage activity interference

Advanced prostate cancer particularly refers to prostate cancer that has not been successfully treated by surgery, chemotherapy, radiation and/or androgen suppressant(s).

The same regimen is proposed for the commencement of treatment of other cancers.

The preferred mode of invention without limiting its use or use of pharmaceutical equivalents to those described herein is to use VIOXX (a registered trademark of Merck Co. for a drug formally known as rofecoxib) or CELEBREX (a registered trademark of Searle and Pfizer for a drug formally known as celecoxib) (both referred to as a "COX-2 inhibitor"), in combination with a therapeutic dose of a 3-hydroxy-3-methylglutaryl-Coenzyme-A reductase inhibitor, namely with Mevacor (a registered trademark of Merck Co. for a drug formally known as lovastatin), or ZOCOR (a registered trademark of Merck Co. for a drug formally known as lovastatin) or cholestin (all referred to as "HMG-CoA inhibitor") starting with the minimum recommended starting doses of each drug on the FDA package inserts attached to provisional application 60/245,592 for the treatment of prostate cancer, or the minimum therapeutically effective amount.

1 The invention retards or drives prostate cancer into remission, best illustrated by
2 lowering the Prostate Specific Antigen, the standard measure of prostate cancer activity
3 in the human body.

4 The method of the invention is the step of administering the combination of COX-
5 2 inhibitor and HMG-CoA inhibitor, including lovastatin or simvastatin and rofecoxib or
6 celecoxib, or the combined sequence of steps of sequentially administering the COX-2
7 inhibitor and HMG-CoA inhibitor, including lovastatin and rofecoxib. An alternative of
8 this method of the invention is the combined sequence of steps of sequentially
9 administering the COX-2 inhibitor and HMG-CoA inhibitor, including lovastatin or
10 simvastatin and rofecoxib or celecoxib. Celecoxib may be used in lieu of rofecoxib, and
11 simvastatin in lieu of lovastatin.

12 Another preferred method is the step of administering the combination of COX-2
13 inhibitor, HMG-CoA inhibitor, particularly lovastatin or simvastatin and rofecoxib or
14 celecoxib, along with cystine as a glutathione pathway enhancing and detoxifying
15 compound. An alternative of this method of the invention is the combined sequence of
16 steps of sequentially administering the COX-2 inhibitor and HMG-CoA inhibitor,
17 particularly including lovastatin or simvastatin, and rofecoxib or celecoxib, along with
18 cystine as a glutathione pathway enhancing and detoxifying compound.

19 Also part of the invention is the method of manufacturing a combination of a
20 COX-2 inhibitor and a 3-hydroxy-3-methylglutaryl-Coenzyme-A reductase inhibitor, that
21 is manufacturing a combination of an HMG-CoA inhibitor, including lovastatin or
22 simvastatin, and a COX-2 inhibitor, including rofecoxib or celecoxib. Also part of the
23 invention is the method of manufacturing a combination of a COX-2 inhibitor, a 3-
24 hydroxy-3-methylglutaryl-Coenzyme-A reductase inhibitor, namely manufacturing a
25 combination of lovastatin or simvastatin, and rofecoxib o celecoxib, along with cystine as
26 a glutathione pathway enhancing and detoxifying compound.

27 Thus, the prior discussion reviews one preferred mode of the invention, a COX-2
28 inhibitor and an HMG-CoA inhibitor. Another mode of the invention includes a COX-2
29 inhibitor and an HMG-CoA inhibitor, including rofecoxib or celecoxib and lovastatin or
30 simvastatin and cystine or another glutathione pathway enhancing compound. As ATP
31 and cholesterol synthesis is being affected in the cancer cell, cystine is being used to

1 enhance the immune system competency and assist normal cells, through the glutathione
2 pathway, in maintaining their stability.

3 The combination of a COX-2 inhibitor and an HMG-CoA inhibitor could also be
4 used as an abortifacient.

5 The invention also can utilize one or more of certain additional active agents in
6 combination with the HMG-CoA inhibitor and COX-2 inhibitor, or in combination with
7 the HMG-CoA inhibitor, COX-2 inhibitor, and cystine. The additional active agents can
8 be in a single dosage formulation, or may be administered to the patient in a separate
9 dosage formulation, which allows for concurrent or sequential administration. Examples
10 of additional active agents which may be employed include squalene epoxidase
11 inhibitors, squalene synthase inhibitors, probucal, glycoprotein IIb/IIIa fibrinogen
12 receptor antagonists, and pharmaceutically acceptable salts of those additional active
13 agents which do not interfere with the HMG-CoA inhibitor and COX-2 inhibitor
14 combination and method or with the HMG-CoA inhibitor, COX-2 inhibitor, and cystine.
15 These and pharmaceutically equivalent agents in the same classes are described in the
16 cited Winokur art, PCT Appl. US98/21901, filed 16 Oct. 1998, published as
17 WO99/20110 entitled "Combination Therapy for Reducing the Risks Associated with
18 Cardio and Cerebrovascular Disease" and in Nichtberger, U.S. Pat. No. 6, 136,804, Oct.
19 24, 2000. The therapeutically effective amount to use for these additional active agents
20 is referred to in the just-cited art, can be seen in the Physician Desk Reference (PDR)
21 2001, and may be seen on the package inserts.

22 The instant pharmaceutical combination comprising an HMG-CoA inhibitor in
23 combination with a COX-2 inhibitor and cystine includes administration of a single
24 pharmaceutical dosage formulation which contains both the HMG-CoA inhibitor and the
25 COX-2 inhibitor and cystine, as well as administration of each active agent in its own
26 separate pharmaceutical dosage formulation. A cystine supplement taken at a different
27 time of day may be a separate dose without the HMG-CoA inhibitor or the COX-2
28 inhibitor. Cystine is the suggested glutathione pathway enhancing and detoxifying
29 compound. The amount of cystine to be included in an oral dosage combination is a
30 therapeutically effective amount to reach normal glutathione levels. Such therapeutically
31 effective amount should preferably and initially be 140mg/70 Kg man twice per day.

1 Where separate dosage formulations are used, the HMG-CoA inhibitor and the
2 COX-2 inhibitor can be administered at essentially the same time, i.e., concurrently, or at
3 staggered intervals, i.e., sequentially. Without the cystine, the instant pharmaceutical
4 combination comprising an HMG-CoA inhibitor in combination with a COX-2 inhibitor
5 includes administration of a single pharmaceutical dosage formulation which contains
6 both the HMG-CoA inhibitor and the COX-2 inhibitor, as well as administration of each
7 active agent in its own separate pharmaceutical dosage formulation. The instant
8 pharmaceutical combinations are understood to include all these regimens.
9 Administration in these various ways is suitable for the present invention as long as the
10 beneficial pharmaceutical effect of the HMG-CoA inhibitor and the COX-2 inhibitor are
11 realized by the patient at substantially the same time. Such beneficial effect is preferably
12 achieved when the target blood level concentrations of each active drug are maintained at
13 substantially the same time. It is preferred that the HMG-CoA inhibitor and the COX-2
14 inhibitor be co-administered concurrently on a once-a-day dosing schedule; however,
15 varying dosing schedules, such as the HMG-CoA once per day and the COX-2 inhibitor
16 once, twice or more times per day, is also encompassed herein. In all courses of
17 administration, the therapeutic doses for cystine can be added, and likely necessitate an
18 additional therapeutic dose early in the administration regimen. As much as possible, a
19 single oral dosage formulation is preferred. A single dosage formulation will provide
20 convenience for the patient, which is an important consideration especially for patients
21 who may be in need of multiple medications. Administration of the HMG-CoA inhibitor
22 or COX-2 inhibitor can be by tablet, liquid suspension, or many other pharmaceutically
23 acceptable carriers known by or used by reasonably skilled practitioners in the art of
24 pharmacology or pharmacological manufacturing including by the combinations and
25 methods in the cited Winokur art, PCT Appl. US98/21901, filed 16 Oct. 1998, published
26 as WO99/20110 entitled "Combination Therapy for Reducing the Risks Associated with
27 Cardio and Cerebrovascular Disease," Nichtberger, U.S. Pat. No. 6, 136,804, Oct. 24,
28 2000, Waldstreicher, WO 01/45698, filed 18 December 2000, published June 28, 2001
29 entitled "Combination Therapy for Treating Neurodegenerative Disease." These
30 pharmaceutically acceptable carriers can be adjusted by a person of ordinary skill in the
31 art of pharmaceutical delivery.

1 The active drugs can also be administered in the form of liposome delivery
2 systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar
3 vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol,
4 stearylamine or phosphatidylcholines. The active drugs may also be delivered by the use
5 of monoclonal antibodies as individual carriers to which the compound molecules are
6 coupled. They may also be coupled with soluble polymers as targetable drug carriers.
7 Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxy-propyl-
8 methacrylamide-phenol, polyhydroxy-ethyl-aspartamide-phenol, or polyethyleneoxide-
9 polylysine substituted with palmitoyl residues. Furthermore, the active drugs may be
10 coupled to a class of biodegradable polymers useful in achieving controlled release of a
11 drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and
12 polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters,
13 polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic
14 block copolymers of hydrogels. All of these are described in Nichtberger, U.S. Pat.
15 6,136,804, Oct. 24, 2000.

16 The term "therapeutically effective amount" is intended to mean that amount of a
17 drug or pharmaceutical agent that will elicit the biological or medical response of a
18 tissue, a system, animal or human that is being sought by a researcher, veterinarian,
19 medical doctor or other clinician. A therapeutic change is a change in a measured
20 biochemical characteristic in a direction expected to alleviate the disease or condition
21 being addressed. The term "prophylactically effective amount" is intended to mean that
22 amount of a pharmaceutical drug that will prevent or reduce the risk of occurrence of the
23 biological or medical event that is sought to be prevented in a tissue, a system, animal or
24 human by a researcher, veterinarian, medical doctor or other clinician. In the preferred
25 mode, the prophylactically effective amount is intended to begin with the minimum
26 recommended dose. The term "therapeutic window" is intended to mean the range of
27 dose between the minimal amount to achieve any therapeutic change, and the maximum
28 amount which results in a response that is the response immediately before toxicity to the
29 patient. The term "minimum recommended dose" is that amount either recommended in
30 the package insert for the selected FDA approved drug, or for other substances and
31 compounds, the minimum therapeutically effective amount for a typical patient of the

1 size and weight being treated, meaning that amount sufficient to precipitate a therapeutic
2 change in condition of a patient for the use of the drug or substance alone for conditions
3 it is designed to treat alone. Minimum recommended dose in the context of commencing
4 treatment is also referred as the minimum initial recommended dose and is that amount
5 recommended for patients as the starting dose. Adjustment of dose upward by 10% or
6 "dose being adjusted upward by at least 10% of the previous dose" means increasing the
7 dose by that approximate amount. In some instances the pharmaceutical carrier, or pill
8 may have to be divided, but generally an increase to the next highest dose is acceptable
9 within the therapeutic window. The references in the claims to specific dosages of
10 specific FDA approved drugs are to tablets having those dosages as referenced in the
11 package inserts adopted herein by reference from Prov. Appl. 60/249,592 dated
12 November 17, 2000. The suggested starting dose for cystine is described in this
13 invention as is the suggested starting dose for silymarin and related compounds to
14 silymarin.

15 The dosage regimen utilizing an HMG-CoA inhibitor in combination with COX-2
16 inhibitor is selected in accordance with a variety of factors including type, species, age,
17 weight, sex and medical condition of the patient; the severity of the condition to be
18 treated; the route of administration; the cardiac, renal and hepatic function of the patient;
19 and the particular compound or salt or ester thereof employed. Dosages in all events
20 should be limited to the therapeutic window. Since two different active agents are being
21 used together in a combination therapy, the potency of each of the agents and the
22 interactive effects achieved by combining them together must also be taken into account.
23 A consideration of these factors is well within the purview of the ordinarily skilled
24 clinician for the purpose of determining the therapeutically effective or prophylactically
25 effective amount.

26 Discussion of pharmacokinetics and summary of literature:

27 The literature has suggested that an HMG-CoA reductase inhibitor may separately
28 have efficacy toward cancers, and that a selective COX-2 inhibitor may separately have
29 efficacy toward certain cancers, but no literature suggests that the substances be
30 combined to treat cancer.

31 In drawing conclusions concerning the pharmacokinetics, the inventors observe that

1 an intriguing and surprising aspect of the invention, which suggests many of the
2 pharmacokinetics, is that quality of life is not substantially affected by the treatment; the
3 patient is alive, the patient does not die; at the same time, at least in the short term, the
4 cancer is also present albeit repressed in its activity. The consideration of pharmacokinetics
5 attempts to comprehend these combined phenomena.

6 An important aspect of the pharmacokinetics is the selectivity to cancer cells and
7 essentially microadministration of cancer therapy. For instance, this invention proposes
8 to affect ubiquinones in important ways. There is art emerging, subsequent to provisional
9 application 60/263,486, to a pending trial of Ubiquinone under a trade name of Ubigel by
10 Gel-Tec, Drug Facts and Comparisons, 55th ed. 2001 at KU-16 (Publ. by Facts &
11 Comparisons 2000). Ubiquinone or CoQ-10 administration, in itself, is not likely have
12 the benefits of the present invention because it is proposed to be administered by
13 macroadministration to the entire organism, either orally or intravenously or in the
14 general vicinity of the tumor area.

15 By contrast to such effort at macroadministration, this invention proposes virtual
16 selective-to-cancer microadministration utilizing the body's own metabolic mechanisms
17 and responses. This is a unique aspect of this invention and an important concept behind
18 the invention. The inventors propose that one of the dilemmas of cancer therapy is to
19 deliver the needed dose to the right place and minimize harm when the therapy is not in
20 the right place.

21 The inventors believe that the most optimal treatments involve the utilization of
22 the biochemical physiologic machine of the body, and preferably of the individual cell, to
23 construct, manufacture and adjust the individual cell chemistry to achieve the desired
24 object: in the case of the cancer cell or other afflicted and undesired cell, to disrupt its
25 mechanisms of replication, primarily by focusing on the energy mechanism of the cell
26 with the corollary result of interfering with membrane synthesis and cell replication, and
27 in many instances, as the cell struggles to reach homeostasis, inducing apoptosis.

28 In sum, by interfering with the cyclooxygenase pathway, particularly important in
29 the formation of prostaglandins, and thus in the cell-signaling mechanism critical for
30 replication of cancer cells, by directly interfering, using an HMG-CoA inhibitor, namely
31 lovastatin, with polyisoprenoid formation and disorienting the feedback regulation system

1 in that formation cycle, and later in that cycle, by utilizing a COX-2 inhibitor, preferably
2 rofecoxib, to further inhibit the formation of cholesterol, the invention renders cancer
3 cells vulnerable to poor replication and subject to bodily defenses, thus slowing the
4 cancer activity, and in the instance of prostate cancer, lowering the PSA of the patient
5 while destroying cancer cells.

6 *The COX-2 inhibitor and the cyclooxygenase-prostaglandin pathway*

7 The COX-2 inhibitor interferes with the operation of the cyclooxygenase cycle
8 from which are generated prostaglandins critical in cell division chemistry. Direct
9 inhibition occurs of the synthesis of COX-2, a precursor of prostaglandins. Biochemistry,
10 Geigy Scientific Tables, Book 4, ed. by C. Lemtner, published by Ciba-Geigy (1986)
11 ISBN -0-91-4168-53-3, Lib. Cong. Cat. No. 81-70045 pp. 25-27 attached to Prov. Appl.
12 60/245,592, the text of which attachment is adopted by reference herein). This effect has
13 been discussed in the literature. Fosslien, "Biochemistry of Cyclooxygenase (COX)-2
14 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin. Lab. Sci.
15 37(5): 431-502 (November 2000). As also previously referenced, COX-2 inhibitors were
16 reported to be inhibiting certain cancers, particularly familial adenomatous polyposis.
17 See, 319 (7218) British Medical Journal 1155 (Oct. 30, 1999). COX-2 inhibitors, in that
18 instance, celecoxib, a COX-2 inhibitor manufactured by G.D.Searle, and sold under the
19 brand name Celebrex, had caused a reduction in adenomatous polyps which are a virtual
20 guarantor of cancer of the colon if left untreated. Cyclooxygenase-2 had been implicated
21 in colorectal cancer and colonic tumorigenesis. See, "The Relationship Between
22 Cyclooxygenase-2 Expressions and Colorectal Cancer", 282(13) J. Amer. Med.
23 Ass'n:1254-1257 (Oct. 6, 1999).

24 Both celecoxib and rofecoxib are suggested to have similar effects. See Vol.
25 56(2) Amer. J. of Health-System Pharmacy: 106-107 (Jan. 15, 1999).

26 One of the clear benefits of the selective COX-2 inhibitor is that COX-1
27 isoenzymes have what has been characterized as having general housekeeping functions
28 generally ameliorative to bodily health. Fosslien, "Biochemistry of Cyclooxygenase
29 (COX)-2 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin.
30 Lab. Sci. 37(5): 431-502 (November 2000). Aspirin, a classic COX inhibitor, also
31 inhibits COX-1, thereby achieving anti-inflammatory effect, for which aspirin is well-

known, at the cost of beneficial aspects of COX-1 isoenzymes. Thus, a COX-2 inhibitor that is selective is important in the invention.

A selective COX-2 inhibitor is important to this cancer management invention, but as the literature indicates, does not provide a comprehensive answer nor a comprehensive cancer response.

The COX-2 inhibitor and angiogenesis

In mice, a COX-2 inhibitor, NS398, was reported to inhibit angiogenesis of a prostate cancer specimen in vivo. Liu et al, "Inhibition of Cyclooxygenase-2 suppresses Angiogenesis and the Growth of Prostate Cancer in Vivo," 164 J. of Urology 820-825 (September 2000) at 820.

Inhibition of cholesterol synthesis by COX-2 inhibitor and HMG-CoA inhibitor:

In viewing the biochemical cycle through which the formation of polyisoprenoids occurs, there are a series of intermediates. See, Biochemistry, Geigy Scientific Tables, Book 4, ed. by C. Lemtner, published by Ciba-Geigy (1986) ISBN -0-91-4168-53-3, Lib. Cong. Cat. No. 81-70045 pp. 25-27, 142-147 (attached to Prov. Appl. 60/245,592, the text of which attachment is adopted by reference herein). A key end product of the biochemical cycle of formation of polyisoprenoids is cholesterol. In order for a cell to replicate successfully, the entire cholesterol cycle must be functioning properly and cholesterol is especially critical to membrane stabilization, a necessary ingredient for successful cancer cell replication.

The "early" cholesterol pathway: Acetyl CoA to mevalonate

Examining the intermediates in the polyisoprenoid formation cycle carefully, beginning with Acetyl-CoA, the next intermediate is 3-Hydroxy-3-methylglutaryl-CoA ("HMG-CoA"). There is a feed back regulation mechanism immediately after this intermediate before transition occurs to the next intermediate: Mevalonate. Salway, Metabolism at a Glance, 88-89 (Blackwell Science 2nd ed. Oxford 1999). The invention proposes to use lovastatin as an HMG-CoA reductase inhibitor. An HMG-CoA reductase inhibitor interferes in the polyisoprenoid formation cycle, and particularly interferes with cell wall synthesis, thereby interfering with a necessary construct of cancer replication. Because ATP cycle intermediaries are juxtaposed to the HMG-CoA feedback mechanism, and ATP and ATP cycle intermediaries are apparent in transition steps of

1 biosynthesis of cholesterol subsequent to the Mevalonate intermediate, the effect of a
2 cancer cell starved of necessary cholesterol is to biochemically invite increased
3 production of intermediaries in the transition from mevalonate to cholesterol, and to
4 biochemically invite increased production of HMG-CoA, whose biosynthesis is being
5 inhibited. Such increased production draws on the ATP and ATP cycle intermediaries in
6 the cancer cell.

7 The later cycle: squalene to cholesterol synthesis

8 Continuing examination of the polyisoprenoid formation cycle, after the
9 Mevalonate intermediate, the cycle continues with the formation of isopentenyl
10 diphosphate, and then farnesyl diphosphate. Three intermediate products emerge after
11 the farnesyl diphosphate intermediary: squalene, dolichols and ubiquinone. Salway,
12 Metabolism at a Glance at 88-89, (Blackwell Science 2nd ed Oxford 1999).

13 A second effect cooperates with the HMG-CoA inhibitor to exacerbate the energy
14 drain on a cancer cell. This collateral effect is additional to the effect of a COX-2
15 inhibitor on the cyclooxygenase cycle. While the HMG-CoA inhibitor has decreased the
16 production of the subsequent intermediates to farnesyl pyrophosphate, the COX-2
17 inhibitor, because of the active electron field substituents, also interferes in a way not
18 discussed in the literature with the normal biochemistry of squalene to cholesterol
19 synthesis. Squalene transitions through a complex series of intermediates to cholesterol.
20 This interference in the biosynthesis pathway subsequent to squalene synthesis further
21 disables the cell division chemistry of a cancer cell and leaves it vulnerable to apoptosis.
22 Notably, the transition states from squalene to cholesterol between intermediaries depend
23 on critical inputs of ATP cycle chemicals, including NADP and NADPH. Salway,
24 Metabolism at a Glance at 88-89, Blackwell Science 2d ed 1999). A COX-2 inhibitor
25 interferes with, but does not appear to stop, synthesis of certain of these intermediaries.
26 This either results in insufficient cholesterol for cancer cell replication or results in
27 introduction of further drain on the ATP cycle chemicals to produce the desired
28 cholesterol critical for cell replication. This drain on the ATP cycle is beyond the stresses
29 already imposed by the HMG-CoA inhibitor. As the replicating cell has further need for
30 cholesterol, further energy is diverted from the cell.

31 The “middle” of the cholesterol synthesis cycle: Farnesyl Pyrophosphate and

ubiquinones

A corollary effect of the partial inhibition of the production of cholesterol from squalene and the triggering of increased production of farnesyl pyrophosphate is that relatively more ubiquinones are produced which are not being inhibited in the same manner as the squalene to cholesterol synthesis is inhibited.

Ubiquinones are key participants in the Q cycle in mitochondrial respiration. With the relative overproduction of ubiquinone that occurs in order to attempt to produce the requisite cholesterol for cell replication, one of two effects, or both effects, occur on mitochondrial respiration.

The replicating cancer cell either comes under osmotic pressure to decrease the concentration of ubiquinone, or the increased ubiquinone concentration changes the electron transport mechanism in the inner membrane of the mitochondria. If the cell admits fluid to stabilize the ubiquinone concentration, the cell must normally change size or shape to do so. Ellerby et al, Measurement of Cellular Oxidation, Reactive Oxygen Species, and Antioxidant Enzymes during Apoptosis, 322 Method in Enzym. 413 (Academic Press 2000), Bortner, Volume Regulation and Ion Transport during Apoptosis, 322 Method in Enzym. 421 (Academic Press 2000).

If the increased ubiquinone concentration changes the electron transport mechanism, the predicted effect is that there is a change in electron transfer from Complex 1 toward Complex 3. See Metabolism at a Glance, J.G. Salway, p. 12-15 (Blackwell Science Ltd., Oxford and London, 2nd ed. 1999).

Simultaneous to the ubiquinone effect, giving attention to both the COX-2 inhibitor with the hydrophilic and lipophilic substituents referred to earlier in this specification and the chemical potential of the unpaired electrons on the first and second substituents, the electrochemical potential and gradient between the matrix side of the membrane and the opposite side membrane is changed, which affects the proton pump and migration of H⁺ ions and in turn interferes with ATP synthesis. The likely reason is one of several, or a combination of several reasons. The COX-2 inhibitor, by changing the electrochemical gradient and potential across the membrane inhibits the potential need for ATP synthesis. Further, the electron attraction to the H⁺ cations on the matrix side, likely from the O=S=O bond in rofecoxib (or celecoxib), either slows the cation,

1 potentially bonds and neutralizes them, or if an excess of electrons pushed by the
2 ubiquinone shuttle from complex II to complex III encounters the cations, they
3 potentially neutralize the H⁺ cations.

4 The cancer cell has an opportunity to again change the concentration to proper
5 levels, but another osmotic pressure is generated. Any disruption in ion transport that
6 produces excess cytochrome would either be potentially fatal to the cell, or require yet
7 another osmotic effect. Bortner suggests a volume loss or movement of ions is associated
8 with cell apoptosis. "Cell volume is normally controlled within narrow limits." Bortner,
9 322 Methods in Enzym. 422. Ellerby associates any change in cell size as either a
10 coincident event to apoptosis or a precursor to completion of apoptosis phases. Ellerby,
11 322 Methods in Enzym. at 413-415. Bortner proposes the thesis that "When cells are
12 placed in a hypertonic environment, shrinkage occurs because of the loss of osmotically
13 obligated water. However, over a period of time diverse cell types compensate for the
14 volume loss by activating a regulatory volume increase (RVI) response. This response
15 allows for an influx of ions, with the concomitant movement of water into the cells to
16 achieve a near-normal size." Bortner, 322 Methods in Enzym. 422. Thus, there is
17 movement of osmotically obligated water from the cell [or to the cell] to achieve a near
18 normal cell size. If not successful, excess cytochrome has been implicated in the
19 generation of caspases which often lead to cell apoptosis. Ellerby, 322 Methods in
20 Enzym. 413-415.

21 Thus, the novel combination for retarding cancer does so in part by producing
22 osmotic stress selectively in cancer cells, and in part by interfering with membrane
23 synthesis in cancer cells. Movement of any osmotically obligated fluid has a corollary
24 effect of also speeding into replicating cells potentially detrimental biochemicals from the
25 body's own immune system. Another corollary of any change in electrochemistry in the
26 area of the matrix or the size of the cell is damage to ion transport channels, the blockage
27 or overexpansion of which ion transport channel is often fatal to the cell. Ellerby, 322
28 Methods in Enzym. 413-421, Bortner, 322 Methods in Enzym. 421-433. The result of
29 mitochondrial respiration uncoupling has been observed in conjunction with non-
30 steroidal anti-inflammatory drugs. Fosslien, "Biochemistry of Cyclooxygenase (COX)-2
31 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin. Lab. Sci.

37(5): 431-502, pp. 453-455 (November 2000).

Since cancer replication is very sensitive to ATP cycle disruptions, the effect is to divert cell energy “unnecessarily” to attempting to overcome the effect of the HMG-CoA inhibitor and the COX-2 inhibitor and starve the cancer cell of necessary energy resulting in cytotoxic effect, apoptotic effect, or inhibition of replication.

Selectivity to cancer cells as a result of anaerobic function of cancer cells

The invention, either in the preferred mode of lovastatin and rofecoxib, or the alternative preferred mode of lovastatin, rofecoxib and cystine, takes advantage of the increased ratio of anaerobic to aerobic functionality of a cancer cell compared to that ration in a normal cell. In the process of replication and mitosis, the growth rates of cancers parallel their level of differentiation and the relative number of their cells in mitosis. Mitoses are more abundant in the anaplastic rapidly dividing variants, meaning in the cancer cells that are creating “clones” of each other by cell division and replication. In most cancers that are associated with an increased number of mitoses and growth rate of cells, such proliferative activity results from the apparent loss of regulatory mechanisms apparent in normal cells. Cancer cells, without these regulatory mechanisms, are so engaged in the mitosis process with its significant energy demands, that both aerobic energy generation and anaerobic energy generation mechanisms are utilized. Nelson and Cox, Lehninger, Principles of Biochemistry (3rd ed. 2000) at 541.

In a normal cell, the combination of glutathione and internal cell biochemical controls enable an efficient disposition of cell wastes. In a cancer cell, the anaerobic processes of the cell to meet the cell’s energy demands result in use of glycolytic mechanisms even in the presence of what would be adequate oxygen supplies in a normal cell. The increased glycolytic processes, particularly the anaerobic processes, generate relative more waste product such as CO₂ and lactic acid. Metabolism at a Glance, J.G. Salway, p. 32-33, 68-69 (Blackwell Science Ltd., Oxford and London, 2nd ed. 1999). Moreover, the COX-2 inhibitor shifts the reaction equilibrium to promote a higher concentration of arachidonic acid. Biochemistry, Geigy Scientific Tables, Book 4, ed. by C. Lemtner, publ. by Ciba-Geigy (1986), p. 25-27; . Fosslien, “Biochemistry of Cyclooxygenase (COX)-2 Inhibitors and Molecular Pathology of COX-2 in Neoplasia,” Crit. Rev. in Clin. Lab. Sci. 37(5): 431, 433 (November 2000). Such relatively acidic

1 environment in the cancer cell interferes with the functionality of the glutathione pathway
2 which pathway is less efficient in an acidic environment.

3 Classic biochemistry indicates that the concentration of glutathione will fall in a
4 more acidic environment such as the relatively more acidic cancer cell. Glutathione is
5 gamma-Glu-Cys-Gly. The COO⁻ ion on the end of the chain will be more present and a
6 more favored species in the less acidic environment of the normal cell.

7 The glutathione functionality is important in reducing reactive oxygen species to
8 relieve subsequent oxidative stress which is deleterious to any cell. The effect in the
9 cancer cell of the relatively reduced glutathione functionality and generation of increased
10 wastes from increased and unregulated glycolysis is to either cause a slowing of the
11 processes leading to waste production, thereby slowing replication, or to cause a change
12 in osmolarity of the cell which is normally offset by increased water and a corresponding
13 change in cell size. By contrast, in normal cells, an enhancement in relief of oxidative
14 stress occurs, as well as maintenance of full functionality, thereby strengthening the
15 immune system competency and total body system.

16 Another accomplishment of the invention not suggested by the literature is to
17 utilize cystine to ameliorate the negative renal, hepatic and gastric effects of COX-2
18 inhibitors and HMG-CoA inhibitors, both as a combination and individually.
19 Unfortunately, like many non-steroidal anti-inflammatory (NSAIDs), the COX-2
20 inhibitors are felt to cause a range of gastrointestinal problems. This amelioration by the
21 invention of negative renal, gastric and hepatic effects is accomplished by cystine,
22 especially in a glutathione deficient patient.

23 The avoidance of a glutathione deficiency steers the patient to have a higher Th-1
24 response to Th-2 response ratio than the patient would have with any glutathione
25 deficiency. Peterson, J. et al, "Glutathione levels in antigen-presenting cells modulate
26 Th1 versus Th2 response patterns," Vol 95(6), Proceedings Nat'l Acad. Sci. USA p.
27 3071-76 (Mar. 17, 1998). This ameliorates negative gastrointestinal hepatic and renal
28 effects. Another article, discussing 5-HETE and its association with prostate cancer,
29 suggests that N-acetyl cysteine in the invention would not be efficacious. Miller et al, ,
30 "5-HETE Congeners as Modulators of Cell Proliferation," Bioorg. Med. Chem. Ltr.
31 10(17): 913-916 (Sep. 4, 2000).

1 The second and unexpected enhancement is independent of, but corollary to, the
2 combination of the COX-2 inhibitor and HMG-CoA inhibitor. Though no source is cited,
3 Fosslien suggests that antioxidants such as TROLOX also inhibit COX-2 induction:
4 "Inhibitors of COX-2 induction are tumor suppressor protein p53, estrogen, and
5 antioxidants such as Trolox (N-acetylcysteine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-
6 carboxylic acid), PDTC, and U75006" Fosslien, "Biochemistry of Cyclooxygenase
7 (COX)-2 Inhibitors and Molecular Pathology of COX-2 in Neoplasia," Crit. Rev. in Clin.
8 Lab. Sci. 37(5): 431, 433 (November 2000). TROLOX is not practical for combating
9 cancer in mammals because it is an extremely powerful anti-oxidant and potentially
10 toxic. In this invention, a more specific anti-oxidant that affects the glutathione pathway
11 and which will have additional COX-2 inhibition characteristics is used. See Fosslien,
12 Crit. Rev. in Clin. Lab. Sci. 37(5): 431 (Nov. 2000)..

13 The correlative effect is that the invention takes advantage of the very "strengths"
14 of the vigorously metastasizing cancer whose strengths weaken the cancer cell's response
15 to cystine and the glutathione pathway because of the cancer cell's Gompertzian growth
16 characteristic.

17
18 *Lovastatin, its interaction with a selective COX-2 inhibitor and isoprostanes and the*
19 *lipxygenase pathway.*

20
21 The cited article entitled, "Caspase-7 is Activated During Lovastatin Induced
22 Apoptosis of the Prostate Cancer Cell Line LNCaP" 58(1) Cancer Research: 76-83
23 (1998), and a second article, Lee et al, "Inhibition of the 3-hydroxy-3methylglutaryl-
24 coenzyme A reductase pathway Induces p53-independent Transcriptional Regulation of
25 p21 (WAF1/CIP1) in human prostate carcinoma cells", 273(17) J. Biol. Chem.:10628-23,
26 (1998), reported that lovastatin had therapeutic value in treating prostate cancer. Patients
27 to whom were administered lipid lowering/modifying drugs such as lovastatin were
28 suggested to be more cancer-free than those using bile acid-binding resins. See, 3-
29 Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors and the Risk of Cancer: A
30 Nested Case-Control Study, 160(5) Archives of Internal Med: 2363-2368 (2000).

31 Lovastatin can be predicted to have another cooperative effect with rofecoxib

1 with respect to cancer, especially prostate cancer. There is strong evidence that oxidative
2 stress and subsequent free radical damage is very important in prostate cancer. Chung et
3 al, Prostate Cancer: Biology, Genetics and the New Therapeutics, "Chemoprevention of
4 Prostate Cancer" by Brooks and Nelson p. 365-375 at p. 369(Humana Press 2001).

5 *COX-2 inhibitor and the lipooxygenase pathway*

6 In examining the cyclooxygenase pathway, see Biochemistry, Geigy Scientific
7 Tables, Book 4, ed. by C. Lemtner, publ by Ciba-Geigy (1986), p. 25, by application of
8 Le Chatelier's principle, an inhibition of the cyclooxygenase pathway will cause the
9 concentration of arachidonic acid to increase. Such increased concentration will cause an
10 increase in products produced in the lipooxygenase pathway. One of those products is
11 Leukotriene B4. Leukotriene B4 is implicated in lipoperoxidative stress to cells.

12 *The lipooxygenase pathway and isoprostanes*

13 As a cancer cell signals for increased COX-2 expression which is being inhibited,
14 the signal is directed to creation of further arachidonic acid ("AA"). The differentiation
15 from normal cells is that a normal cell is not signaling for more AA to delivery more
16 COX-2 expression. From both COX-2 inhibition and saturation from products of AA
17 in the lipooxygenase ("LPO") pathway, a significant buildup of AA occurs which can be
18 most easily relieved from a redox viewpoint by creation of isoprostanes.

19 Such excess production has implications for the lipooxygenase metabolic
20 pathway. The evidence for this lipooxygenase pathway effect is seen in isoprostanes
21 which are prostaglandin-like compounds which are formed by free radical catalysed
22 peroxidation of arachidonic acid esterified in membrane phospholipids (Neurochem Res
23 2000 Oct;25(9-10):1357-64).

24 Unfortunately for the cancer cell, isoprostanes are indicators of damage to membrane
25 phospholipids. Arachidonic acid (AA) is sterified in the membrane phospholipids, and
26 when oxidized, isoprostanes are the end-product. The peroxidation products are
27 monitored by measuring the isoprostanes and lipid peroxides. For a rapidly dividing
28 cancer cell in which membrane synthesis is critical, the increase in arachidonic acid and
29 its potential damage to membrane phospholipids has negative implications for replication
30 success. The rise in isoprostane levels shows that oxidation of excess arachidonic acid is
31 occurring. This is one mechanism for the damage from excess arachidonic acid that may

1 be seen with the use of the COX-2 inhibitors and contributes to explaining the toxic
2 effect of a COX-2 inhibitor, especially in rapidly dividing cells. However, presence of
3 the isoprostane in the blood or urine would signal an upper limit has been reached of the
4 COX-2 inhibitor above which the risk of kidney or liver damage may increase.

5 Lipid peroxidation is best characterized as a series of chain breaking reactions in
6 the lipid bi-layer at the membrane which inhibits the proper growth of proteins. The
7 membrane is rendered more porous and susceptible to degeneration, or to penetration by
8 other molecules in the body's immune system. Analogously, lipid peroxidation by heat
9 occurs in an egg white when heated. In the body, and as is desired in cancer cells, such
10 lipid peroxidation occurs chemically.

11 The HMG-CoA reductase inhibitor simvastatin has been shown to produce
12 positive effects in the endothelial lining of blood vessels even independent of its lipid
13 lowering effects. Animals with high cholesterol diets who exhibited continued high
14 serum cholesterol who were administered simvastatin demonstrated a lower rate of
15 production of F(2)-isoprostanes and thiobarbituric acid-reactive substances (TBARS),
16 markers of oxidative stress, than animals who were not treated with simvastatin and
17 maintained on a high cholesterol diet. *Arterioscler Thromb Vasc Biol* 2001
18 Jan;21(1):122-8). Simvastatin is an analog of lovastatin, which are both statins produced
19 from *aspergillus terreus*.

20 The presence of the HMG-CoA reductase inhibitor may contribute to moderating
21 the effects of lipid peroxidation produced in the normal cells moderating production of
22 isoprostanes.

23 While a protective effective may not seem facially desirable, consideration needs
24 to be made of the selectivity which occurs. The cancer cell metabolic pathways which
25 result in the higher expression of COX-2 in cancer cells, which the invention proposes to
26 inhibit, suggest that cancer cells utilize COX-2 in a meaningful way, a conclusion
27 supported by the apparent partial efficacy of COX-2 inhibitors against cancer. In order to
28 obtain COX-2, cancer cells have a signaling system to stimulate the precursor of COX-2,
29 which is arachidonic acid. Normal cells which do not have a similar need for COX-2
30 apparently do not have such a signaling system.

31 For a cancer cell which under normal replication conditions will experience a

1 more rapid genesis of lipid peroxidation products from membrane synthesis, the inventors
2 surmise that the partial protective effect of a statin to slow the rise in isoprostane levels is
3 selectively insufficient to protect the cancer cell from excess arachidonic acid, while
4 acting protectively in normal cells. As a corollary, whatever offsetting benefit the statin
5 may have against the lipooxygenase pathway products is not sufficient to overcome either
6 the toxic effects of excess arachidonic acid, nor to offset the cholesterol synthesis
7 inhibition occurring in the cholesterol synthesis pathway with respect to production of
8 mevalonate and occurring with respect to excess geraniol as a result of interference with
9 squalene conversion to cholesterol.

10 Thus, there is a selective effect of increased toxic metabolites when a COX-2
11 inhibitor is administered as evidenced by increased isoprostane levels, with end products
12 that have primary toxicity to cancer cells from excess lipid peroxidation and the LTEB4.
13 Biochemistry, Geigy Scientific Tables, Book 4, ed. by C. Lemtner, publ by Ciba-Geigy
14 (1986), p. 25-27, 142-147.

15 Testing of isoprostanes and TBAR's can be used to determine if excessive
16 amounts of lovastatin or any statin are being used and as an indicator of the level of
17 lipooxygenase peroxidation effects.

18 Another product that can result from increased arachidonic acid is 5-HETE which
19 has been implicated in prostate cancer. Miller et al, "5-HETE Congeners as Modulators
20 of Cell Proliferation," Bioorg. Med. Chem. Ltr. 10(17): 913-916 (Sep. 4, 2000). It is
21 poorly disposed of. However once saturated, it will cause increased arachidonic acid
22 buildup if arachidonic acid buildup is being artificially stimulated such as by a COX-2
23 inhibitor. Further evidence of this effect of increased AA concentration is shown
24 from experiments with γ -linoleic acid which is the precursor of arachidonic acid through
25 the formation of dihomo- γ -linoleic acid ("Metabolism at a Glance", Salway, 2nd edition,
26 BlackWell Sciences, UK pg. 86). Conjugated linoleic acid (CLA) is prone to oxidation,
27 and it has been suggested that increased oxidation of lipids may contribute to an anti-
28 tumorigenic effects of this agent. Clin Sci (Colch) 2000 Dec;99(6):511-6. There,
29 researchers followed levels of 8-iso-prostaglandin F(2alpha) (8-iso-PGF(2alpha)), a
30 major isoprostane, and of 15-oxo-dihydro-PGF(2alpha), a major metabolite of
31 PGF(2alpha), (collectively referred to as isoprostanes) and tested their levels, as

1 indicators of non-enzymic and enzymic arachidonic acid oxidation respectively after
2 dietary supplementation with CLA in middle-aged men (mean age 53 years) with
3 abdominal obesity for 1 month in a randomized controlled trial. Thus, the addition of
4 CLA to the diet of people undergoing metabolic cancer therapy with a Hmg-CoA and a
5 COX-2 inhibitor would result in an enhanced effect by increasing the lipid oxidation
6 effect of the isoprostanes, and shows the creation of excess arachidonic acid has
7 antitumorigenic effect as predicted by the inventors.

8 Using the isoprostane levels as indicators, the treatment dose of the COX-2
9 inhibitor can be maximized to give the maximum tolerated dose for use in cancer therapy
10 without creating excessive systemic toxicity. More lipid oxidation activity indicates
11 increased oxidative stress, usually a characteristic of cancer activity. A long-term falling
12 level of isoprostanes will mean for COX-2 expressing cancers that there is relatively less
13 cancer risk. An ELISA test for isoprostane level is available from Cayman Chemical
14 Company, 11800 E. Ellsworth Rd., Ann Arbor, Michigan.

15 For a membrane-impaired cancer cell, receptors and transport molecules for
16 materials needed for cell survival tend to be overloaded and the cell does not function
17 properly, much less have much chance of replicating accurately with an intact membrane.
18 Additionally, in this invention, the shift in concentration caused by excess ubiquinones
19 toward semiquinone triggers increased lipid peroxidation. Nohl, "Antioxidant-derived
20 prooxidant formation from Ubiquinol," Free Radical Biol. Med. 25(6): 666-675 (Oct.
21 1998). While the statin can ameliorate the tendency to lipid peroxidation, which is why a
22 lower dose is preferred, it need only be sufficient to impair cholesterol synthesis, and
23 there remain sufficient lipid peroxidants to damage cancer cells while normal cells are
24 slightly protected.

25 The presence of ubiquinones in normal cells with adequate glutathione does not
26 materially change their characteristics; however in cancer cells, the excess ubiquinones
27 in combination with the already nascent tendency to express lipid peroxidation
28 sufficiently weakens the cells to expose them to immune system attack, a tendency
29 not overcome by the presence of glutathione which is less active in the more anaerobic
30 environment of a cancer cell.

31 *Lovastatin and its inhibition of farnesyl pyrophosphate and*

1 *geranylgeranylpyrophosphate*

2 Lovastatin has another inhibitory effect which has implications for both
3 cholesterol synthesis, ubiquinone concentration, and farnesyl pyrophosphate
4 concentration. "Lovastatin, an HMG-CoA reductase inhibitor that inhibits the
5 biosynthesis of farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GPP), is
6 used routinely as a positive control for inhibition of processing of both
7 geranylgeranylated and farnesylated proteins [citations omitted]." A. Vogt et al, "A Non-
8 peptide Mimetic of Ras-CAAX: Selective inhibition of Farnesyl Transferase and Ras
9 Processing," 270(2) J. Biol. Chem. 660-664 (2000). In addition to additional direct
10 cholesterol inhibition, Salway, Metabolism at a Glance at 88-89 (Blackwell Science 2nd
11 ed. 1999), the effect of any FPP inhibition is to directly inhibit production of dolichols,
12 which has implications for dolichol phosphate which affects messenger RNA
13 transcription. Since cancer cells are attempting to replicate, a selective effect on cancer
14 cells by affecting messenger RNA is achieved. Lehninger on Biochemistry at 1059, 3rd
15 ed. GPP inhibition likely has the same effect as post-lanosterol cholesterol cycle
16 inhibition in that additional energy must be used to overcome inhibitory effects. The
17 Vogt article also notes that cysteine is important in ras oncogene activation. This teaches
18 away from the benefits of glutathione pathway protection, but the inventors suggest that
19 the combination of diversion of glutathione pathway resources to stabilize other
20 adversely affected metabolic pathways of a cancer cell is likely sufficient in combination
21 with FPP and GPP inhibition to interfere with cell replication. What FPP is generated
22 will be diverted to enhance cholesterol synthesis making it less available for ras oncogene
23 activation in conjunction with cysteine.

24 *Lipid peroxidation and reactive oxygen and nitrogen species*

25 The article entitled "Reactive Oxygen and Nitrogen Species: Efficient, Selective,
26 and Interactive Signals During Intercellular Induction of Apoptosis; Georg Bauer,
27 Abteilung Virologie, Institute for Medizinische Mikrobiologie und Hygiene, Universität
28 Freiburg, D-79104 Freiburg, Germany; Anticancer Research 20: 4115-4140 (2000)
29 contains a comprehensive discussion of the interplay of reactive nitrogen species and
30 oxygen species with apoptosis. See also, Bolanos, Nitric Oxide, Mitochondrial Function
31 and Excitotoxicity, Methods Findings Exp. Clin Pharmacol, 2000, 22(6): 375-77. The

1 Bauer article sets out a series of chemical equations related to processing of reactive
2 oxygen and nitrogen species.

3 The issue is what about a selective COX-2 inhibitor, the overproduction of
4 ubiquinones, and the interference with mitochondrial respiration, assuming an adequate
5 supply of glutathione, enables the invention to be effective. We have already recognized
6 that additional energy will be needed to generate cholesterol both because of HMGCoA
7 inhibition and squalene-to-cholesterol synthesis inhibition.

8 The answer from the Bauer article focuses on the tendency of excess NO and OH
9 species, particularly in their free radical forms, to accelerate lipid degeneration.

10 As stated previously, lipid peroxidation is best characterized as a series of chain
11 breaking reactions in the lipid bi-layer at the membrane which inhibits the proper growth
12 of proteins. The membrane is rendered more porous and susceptible to degeneration, or
13 to penetration by other molecules in the body's immune system.

14 In an article entitled "Antioxidant-Derived Prooxidant Formation from
15 Ubiquinol...", Nohl et al, Free Radical Biol. Med. 25(6): 666-75 (Oct. 1998) set forth
16 that "Our studies on the antioxidant activity of ubiquinol in peroxidizing lipid membranes
17 demonstrate the existence of ubisemiquinone (SQ•) as the first reaction product of
18 ubiquinol. A reaction of SQ• derived from the localization allows an access of protons
19 and water from the aqueous phase to SQ• [,] a prerequisite earlier found to trigger
20 autoxidation. Superoxide radicals emerging from this fraction of autoxidizing SQ• form
21 H₂O₂ by spontaneous dismutation. SQ• not involved in autoxidation may react with
22 H₂O₂. Transfer of the odd electron to H₂O₂ resulted in HO• and HO- formation by
23 homolytic cleavage. An analogous reaction was also possible with lipid hydroperoxides
24 which accumulate in biological membranes during lipid peroxidation. The reaction
25 products emerging from this reaction were alkoxyl radicals. Both HO• and alkoxyl
26 radicals are strong initiators and promoters of lipid peroxidation." Id. Abstract to
27 "Antioxidant-Derived Prooxidant Formation from Ubiquinol...", Nohl et al, Free Radical
28 Biol. Med. 25(6): 666-75 (Oct. 1998).

29 To summarize the important postulates of Bauer with respect to their
30 interrelationship with this invention, first, •NO in the presence of O₂•- forms

1 peroxynitrite ONNO-. This is not stable. Interestingly, this peroxynitrite is not a free
2 radical. However, in the acidic environment of a cancer cell, there is a propensity to form
3 "the instable peroxynitrous acid.... Peroxynitrite has the potential for lipid peroxidation
4 (no formula shown [in the article]). Id. at 4119. "Singlet oxygen, formed after
5 interaction of hydrogen peroxide and peroxynitrite [f.n. omitted] has an extremely short
6 half-life and has the potential for lipid peroxidation[f.n. omitted]. Nitric oxide, though
7 being a free radical shows a long range of action and rather low toxicity. It inhibits lipid
8 peroxidation and caspases. Interaction of nitric oxide with superoxide anions causes the
9 formation of peroxynitrite, a potent lipid peroxidant and apoptosis inducer." Id. at 4116.
10 There are a series of reactions, several of which involve glutathione.

11 The positive empirical results from the patients on which this invention was tested
12 indicate that peroxynitrite acts as a strong oxidant when increased there is cytokine
13 production. With the increase in ubiquinones causing increased production of
14 superoxide, relatively more of which is available in cancer cells to cause peroxynitrite
15 formation at appropriate pH, the peroxynitrite can cause direct damage to proteins. The
16 second and third reactions discussed are degeneration by homolysis, $\bullet\text{OH} + \bullet\text{NO}_2$, or
17 heterolysis degenerating to $\bullet\text{OH} + \text{NO}^+$. Even the fourth reaction, ONOOH to
18 ONOOH⁺ is troublesome for a cancer cell because of the creation of a more acidic
19 environment.

20 Equally apparent from the equations is the importance of glutathione in
21 detoxification of radical species and prooxidant species such as ONNO-. Glutathione is
22 thought to have a protective effect in a number of instances. However, as postulated,
23 glutathione functions more actively in an anaerobic environment. As a cancer cell's
24 energy needs are stressed by a COX-2 inhibitor, more anaerobic respiration occurs,
25 lowering the pH of the cancer cell slightly, shifting even glutathione reactions away from
26 oxidation to more benign species and generating more free radical damage and
27 accelerating lipid peroxidation. While cancer cells having complete angiogenesis will be
28 less affected by these reactions, the inclination to apoptosis and the degeneration of
29 angiogenic species either as a result of the death of a cell, or the waste of energy in the
30 tumor to generate unutilized angiogenesis both inhibit the cancer cell's growth. Bauer
31 notes that his key reactions occur early in tumor development prior to angiogenesis,

1 Bauer, 20 AntiCancer Research at 4115, a result consistent with the inventors' clinical
2 observation that cancer is not eliminated but retarded or managed by the invention.

3 The presence of ubiquinones in normal cells with adequate glutathione does not
4 materially change their characteristics; however, in cancer cells, the excess ubiquinones
5 in combination with the already nascent tendency to express lipid peroxidation
6 sufficiently weakens the cells to expose them to immune system attack, a tendency not
7 overcome by the presence of glutathione which is less active in the more anaerobic and
8 more acidic environment of a cancer cell.

9 *Metal complex ions and glutathione*

10 Another aspect to consider is that H_2O_2 has a potential rescuing effect for cells to
11 blunt NO mediated apoptosis at high cell density. A primary generator of H_2O_2 is
12 glutathione reactions which in a normal cell environment remove hydroxyl radicals, and
13 nitric oxide radicals. In conjunction with metal ions, particularly copper, zinc and
14 magnesium, in glutathione competent cells, the H_2O_2 breaks down into water. As
15 explained by Bauer, cells are in a sense rescued from apoptosis in that situation. In cells
16 not so equipped, which would include a number of cancer cells in a tumor, more
17 hydroxyl radicals are generated, and there is not a rescue from apoptosis. The fact that,
18 as explained by Bauer, H_2O_2 is a far-ranging species that can intercept NO species far
19 from a cell membrane may explain for small cell cancers, where intercellular range is less
20 of an issue, the relatively toxicity and tumorigenicity of those cancers where the range of
21 operation is less of a factor in what self-protective mechanisms the body has to battle the
22 cancer. The presence of HOCl cannot be ignored which Bauer believes interacts with
23 H_2O_2 to generate non reactive molecules such as oxygen, water, chloride anions and
24 protons. Bauer, 20 AntiCancer Research 4115-4140, generally.

25 Notably, however, Bauer remarks that the speed of reaction is not significant
26 unless reaction number 3 [$HOCl + H_2O_2$ to $O_2 + H_2O + Cl^- + H^+$] is blocked by SOD
27 which is more likely to occur in the COX-2 inhibitor affected cancer cell because of the
28 shift in electron concentration generating more potential O_2^- . Bauer, 20 AntiCancer
29 Research 4118-19. As the kinetics for this reaction to occur become more favorable,
30 SOD, which has been stably attached to Mn, Zn or Cu, is detached as the reaction
31 proceeds and the SOD performs its catalytic function. The resultant free radical metal

1 ion generated, in the presence of HOCl, accelerates lipid peroxidation. Bauer, Anticancer
2 Research 20: 4115-4140 (2000) at 4118-19.

3 Glutathione (GSH), a critical element in immune system function, unquestionably
4 has some positive effects for the cancer cell because it can scavenge free radicals. Yet
5 this is needed in all cells. Glutathione does have a favorable effect on cancer cells
6 through its protection of the disulfide bridges. Protection of disulfide bridges inhibits
7 lipid peroxidation therefore protecting protein structure, particularly tertiary and
8 quaternary structures. "Glutathione probably helps maintain the sulfhydryl groups of
9 proteins in the reduced state and the iron of heme in the ferrous (Fe^{2+}) state, and it serves
10 as a reducing agent for glutaredoxin in deoxyribonucleotide synthesis (see Fig. 2-37 [in
11 source]). Its redox function is also used to remove toxic peroxides formed in the normal
12 course of growth and metabolism under aerobic conditions: $2\text{GSH} + \text{R-O-O-H} \rightarrow \text{GSSG} + \text{H}_2\text{O} + \text{R-OH}$." Lehninger, Principles of Biochemistry (3rd ed. 2000) at 842. As is
13 apparent from the quotation, any effect on glutathione supply, such as failure to remove
14 toxic peroxides, or lack of presence for deoxyribonucleotide synthesis because of
15 competitive consumption to maintain homeostasis in cancer cells has serious implications
16 for cell division and replication, which is the lifeblood and toxicity of cancer.

17
18 Glutathione, however, will be slightly less present in the acidic environments of
19 cancer cells. Glutathione is gamma-Glu-Cys-Gly. The COO^- ion on the end of the chain
20 will be more present and a more favored species in a less acidic environment. The more
21 acidic environment of anaerobic glycolysis in cancer cells causes a shift to moderately
22 lower relative glutathione concentrations, and consequently less protection from
23 apoptotic free radical reactions.

24 The implications of metal ion reactions and glutathione, as seen in the Bauer
25 equations, Anticancer Research 20: 4118-19 (2000), are that glutathione absorption in
26 stabilizing free radicals to convert them to H_2O_2 has implications in coincidentally
27 affecting the reaction kinetics of superoxide dismutase (SOD) and affecting the metal ion
28 chemical reactions illustrated by Bauer under "M" at Anticancer Research 20: 4118.

29 This invention does not propose to be *prima facie* a cancer cure, but rather a *prima*
30 *facie* cancer manager. The competitive consumption of energy to overcome cholesterol
31 synthesis, to overcome interference with mitochondrial respiration, and the competitive

consumption of GSH to thwart lipid peroxidation, and to rescue cancer cells from reactive oxygen and nitrogen species either weakens existing cells, weakens newly generated cells (which may then undergo self-apoptosis) or inhibits membrane and DNA synthesis or all of these. The inherent characteristics of replicating cancer cells and the necessary anaerobic enhancement to their energy processes enable the invention to selectively attack cancer cells while normal cells and their homeostatic processes can protect the mammalian organism which the inventors desire to preserve. Moreover, the administration of the compounds in the invention enable the organism to achieve the senescence which cancer cells have attempted to elude through a variety of mechanisms that the body in many instances is helpless to resist. The use of HOCl, and the application of NO•- and OH•- is the usual means to achieve senescence, and the invention enables proper operation of that mechanism.

NADPH concentration, COX-2 inhibitors and apoptosis

A corollary effect of the inhibition of creation of cholesterol relates to the shifting of equilibrium toward to squalene and a higher concentration of NADPH+H⁺ as a result of the action of the COX-2 inhibitor. As remarked by Bauer, what is at issue is high speed bursts of adjacent NO/O₂⁻ activity which can damage membranes and cells. The marginal and momentary increase in NADPH +H⁺ has a series of contradictory effects. Exterior to the mitochondria, increased levels of NADPH can be seen to slow reactions in the pentose phosphate pathway, namely in the transition from glucose 6-phosphate to ribulose 5-phosphate. Selective shifts in this pathway affect glucose-6-phosphate, though perhaps only mildly. NADPH concentration shifts also slow the conversion of malate to pyruvate, a precursor to acetyl CoA, a precursor to cholesterol, a possible positive in inhibiting cancer cell membrane synthesis. Another effect is a buildup of lactic acid with concomitant cytotoxic effects for cells unable to tolerate increased acidity. Salway, *Id.* at pp. 49, 60. Salway remarks on this shift indirectly, noting that “during re-feeding after fasting, glucose is metabolized anaerobically to lactate by muscle even though the conditions are aerobic. This is because, immediately after refeeding, the high ratio of acetyl CoA to pyruvate caused the lingering B-oxidation of fatty acids, results in pyruvate dehydrogenase remaining inhibited... Consequently, glucose in muscle is metabolized to pyruvate which is reduced to lactate. Salway, *Metabolism at a Glance* (Blackwell

1 Science Oxford 1999) at p. 60. A similar effect occurs occurs for cancer cells affected
2 by an HMG-CoA reductase inhibitor. The increased acetyl CoA buildup in cancer cells
3 causes increased lactate production. Salway, Id. at 51. That lactate tends to slightly
4 acidify the cancer cell, which has implications in induction of apoptosis. In normal cells,
5 homeostasis is such that an Acetyl CoA imbalance is not toxic on refeeding after
6 starvation because the Acetyl CoA /CoA precursor ratio is not affected.

7 In cancer cells where increased Acetyl CoA has to be present to overcome the
8 inhibition of synthesis of cholesterol, there is a transient increase of acidity, favoring the
9 reaction of peroxynitrite to NO- and OH- apoptotic free radicals.

10 NADPH is also implicated in the presence of NADPH oxidase in the generation
11 of free electrons leading to O₂•- species. As explained by Bauer, these are implicated in
12 induction of apoptosis. In cancer cells demanding cholesterol, as the reactions of
13 intermediates from squalene and lanosterol to cholesterol are slowed by a selective COX-
14 2 inhibitor, there are momentary increases in NADPH. This has apoptotic effects
15 selective to cancer cells as opposed to normal cells.

16 The discussion above, and the article by Bauer, "Reactive Oxygen and Nitrogen
17 Species: Efficient, Selective, and Interactive Signals During Intercellular Induction of
18 Apoptosis," Anticancer Research 20: 4115-4140 (2000), amply confirm and correlate
19 with the observations of Ellerby et al, Measurement of Cellular Oxidation, Reactive
20 Oxygen Species, and Antioxidant Enzymes during Apoptosis, 322 Method in Enzym.
21 413 (Academic Press 2000), Bortner, Volume Regulation and Ion Transport during
22 Apoptosis, 322 Method in Enzym. 421 (Academic Press 2000) regarding the apoptotic
23 cascade that can be triggered by the osmotic pressures on a cancer cell as it struggles to
24 maintain chemical homeostasis. The chemical kinetics and reactions confirm the clinical
25 observations with respect to the invention. On balance, the tendency of the combinations
26 in the invention is to selectively disfavor cancer cells based on the inventor's empirical
27 observations. The inventors also note that the explanation of pharmacokinetics is consistent
28 with the tendency of tumors, once expanded to have a mass of necrotic tissue within them
29 (another complicating factor of cancer), suggesting that glutathione activity,
30 accumulation of wastes and apoptosis are natural mechanisms of cancer cells which the
31 science of this invention attempts to exploit at an earlier stage of cancer cell development

1 in order to manage tumor activity.

2 *Metal complex interactions:*

3 The interaction of nitrous oxide and reactive oxygen species is one of the most
4 important apoptotic triggers in anti-tumor activity. As previously discussed, COX-2 has
5 two interactions with mitochondrial respiration and ATP utilization, one direct and one
6 indirect. The direct interaction is the lipophilic/hydrophilic orientation which can inhibit
7 the F0/F1 channel in complex IV. Salway, *Metabolism at a Glance* at 14-15 (Blackwell
8 Science 2nd ed. 1999). The indirect interaction is the increased relative production of
9 ubiquinone as a result of the inhibition of cholesterol demethylation.

10 Metal ions have the capacity to catalyze, in conjunction with superoxide
11 dismutase (SOD), generation of compounds influential in apoptotic process. Bauer,
12 *Reactive Oxygen and Nitrogen Species: Efficient, Selective, and Interactive Signals*
13 *During Intercellular Induction of Apoptosis*, Anticancer Research 20: 4115-4140 (2000)
14 at 4118. See also, Bolanos, *Nitric Oxide, Mitochondrial Function and Excitotoxicity*,
15 *Methods Find Exp. Clin. Pharmacol.* 2000 22(6): 375-77.

16 Wink and Mitchell, in *Chemical Biology of Nitric Oxide: Insights into*
17 *Regulatory, Cytotoxic, and Cytoprotective Mechanisms of Nitric Oxide*, *Free Radical*
18 *Biol. & Med.* 25(4): 434-456, Sept. 1998, suggest that changes in NADPH oxidase and
19 nitric oxide levels can affect the availability of iron in a cell. This has catastrophic
20 implications for a selectively affected cancer cell. *Id.* at 447.

21 Selective disturbance of metal ion interaction in cancer cells will enhance any
22 probability of apoptosis engineered by other metabolic mechanisms.

23 *Particular efficacy for androgen responsive prostate cancer:*

24 The interference with cholesterol synthesis has a further implication for prostate
25 cancer because cholesterol is a precursor to testosterone which has been shown to be an
26 important contributor to prostate cancer. Androgen suppression is a standard therapy for
27 several lines of prostate cancer, but tends to have time limitations before certain cells
28 become androgen insensitive. *Prostate Cancer: Biology, Genetics, and the New*
29 *Therapeutics* p. 92 and Ch. 19 at 327-340 (Humana Press, Totowa NJ 2001). While the
30 body has other offsetting mechanisms to continue to signal for generation of androgen,
31 there is at least a partial biochemical effect resulting from interference with cholesterol

1 synthesis.

2 The invention is not meant to be limited to the disclosures, including best mode of
3 invention herein, and contemplates all equivalents to the invention and similar
4 embodiments to the invention for humans and mammals and veterinary science.

5 Equivalents include all pharmacologically active racemic mixtures, diastereomers and
6 enantiomers of the listed compounds and their pharmacologically acceptable salts.

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